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EFFECTS OF WHITE CELL FILTRATION, ACD CONCENTRATION AND ROTATION DURING COLLECTION, STORAGE AND CRYOPRESERVATION OF PLATELET CONCENTRATES

BY

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ABSTRACT

The objective of this report was to evaluate the conditions for collection and 24 hour liquid-storage of platelets prior to cryopreservation. Recently there has been a tendency to reduce the amount of anticoagulant used in platelet collection procedures for the comfort of the donor. Plateletpheresis procedures are designed to increase the platelet yield and reduce white cell contamination. These changes could adversely affect the quality and preservation of the plateletpheresis product. In a preliminary study, the effect of leukoreduction of the platelets was addressed. Single donor platelets were prepared from blood drawn into anticoagulant-citrate-dextrose-solution (ACD, formula A) at a ratio of ACD:blood of 1:8 from 14 healthy donors using the Haemonetics V50 Blood Processor with surge. The products were filtered with the Pall leukoreduction filter (LRF) #10 within 30 minutes of collection, after storage at 22°C with rotation for 4 hrs or after storage with rotation at 22°C for 5 days. White cells were counted before and after filtration using propidium iodide staining, a fluorescence microscope and a Nageotte chamber. No differences in white cell removal or platelet recovery were seen following filtration at 0 hr, 4 hr or 5 days. There was a mean of a 3.5 log removal of white cells with a total of $<10^6$ white cells remaining and 90% of the platelets were recovered. No platelet damage was seen as assessed by plasma β-thromboglobulin, thromboxane B_2 level, platelet production of thromboxane A_2, platelet aggregation and recovery from hypotonic stress after filtration at 0 hr, 4 hr and 5 days. The filtration of platelets following storage at 22°C for 5 days was associated with a significant reduction in both MPV and pH. Following these studies, a prospective randomized study was designed to address the effect of anticoagulant concentration, rotation vs. non-rotation during the first 4 hrs of the 24 hr storage period, and filtration vs. non- filtration at 24 hrs prior to freezing on the quality of the product. In each of 5 normal donors, plateletpheresis products were collected with an ACD to blood ratio of 1:8, 1:10, or 1:12 using the Haemonetics V50 with the surge protocol. The products were split equally into two 600 ml polyvinylcloride plastic transfer packs. One was stored at room temperature on a bench (non-rotated) for 4 hrs and the other stored at 22°C on an Eberbach shaker at 140 lateral oscillations per minute (rotated) for 4 hrs. Both were stored at 22°C for the remaining 20 hrs on the Eberbach shaker. The product rotated for the entire 24 hr period (24 hr rotated) was filtered using a Pall LRF #10 filter and frozen in a final concentration of 6% DMSO, while the 4 hr non-rotated-20 hr rotated product was frozen in a final concentration of 6% DMSO without filtration. Electrical impedance methods were used to measure platelet number, volume, and aggregates, white cell counts were measured using the propidium iodide method and plasma thromboxane levels were determined by RIA during the 24 hr testing period, and after thawing and washing the frozen cells. Flow cytometric methods were used to measure platelet aggregates, and platelet derived microparticles by log forward light scatter identified by binding of a glycoprotein (GP) IIIa-specific monoclonal antibody in the baseline peripheral blood of the donor and on the product during the 24 hour storage period. Neither the ACD:blood ratio,

nor the use of rotation during the first 4 h of storage had any significant effect upon: 1) the number of activated platelets (determined by P-selectin), 2) platelet surface GPIb, 3) platelet reactivity *in vitro*, determined by up-regulation of platelet surface P-selectin and down-regulation of platelet surface GPIb in response to thrombin, ADP/arachidonic acid, and U46619 (a stable thromboxane A_2 analogue), and 4) the number of platelet-derived microparticles. An ACD:blood ratio of 1:8 at collection resulted in a minimal number of platelet aggregates as measured by flow cytometry immediately after the preparation of plateletpheresis products, whereas ACD:blood ratios of 1:10 or 1:12 resulted in significantly more aggregates. After 4 h of storage, the number of platelet aggregates increased in 1:8 ACD:blood samples and decreased in 1:10 and 1:12 ACD:blood samples such that the number of platelet aggregates was not statistically different, irrespective of the original ACD:blood ratio and of whether or not there was rotation. After 24 h of storage of rotated plateletpheresis products, the number of platelet aggregates was similar to the 4 h time point for the non-rotated and rotated platelets, and not significantly influenced by the original ACD:blood ratio. In contrast, after 4 h of storage of non-rotated plateletpheresis products followed by rotation for 20 hours, there was virtually complete disaggregation of the 1:8 ACD:blood samples whereas the 1:10 and 1:12 ACD:blood samples had a similar number of aggregates as at the 4 h time point for non-rotated and rotated platelets. ACD:blood collection ratios of 1:10 and 1:12 resulted in lower total platelet counts than the 1:8 ACD:blood ratio, but the trends were not statistically significant. Impedance measurements of mean platelet volume and aggregates did not detect any differences in the 3 study populations. In summary: 1) White cell filtration can be done 24 hrs after collection prior to freezing. 2) Flow cytometry is a sensitive and simple method for the detection of platelet aggregates in platelet transfusion products. 3) With the Haemonetics V50 with surge, platelet aggregates in plateletpheresis products are minimized under the following conditions: a) an ACD:blood ratio at collection of 1:8 rather than 1:10 or 1:12, b) no rotation of plateletpheresis products during the first 4 h of storage.

INTRODUCTION

This study was performed to assess methods for the enumeration of intact platelets, platelet aggregates and platelet microparticles in fresh platelets, platelets stored at 22°C, and cryopreserved platelets. The methods used included phase microscopy, the Coulter electronic impedance method, and flow cytometry using log forward light scatter. Also measured was the platelet aggregation response to agonists in vitro, platelet production of thromboxane B_2 following stimulation with agonists in vitro, platelet surface markers prior to and following in vitro response to different agonists, platelet response to hypotonic stress, and plasma pH, pCO_2, pO_2, thromboxane B_2, and complement C3a.

Single donor platelets were collected from healthy volunteers by a plateletpheresis procedure using the Haemonetics V50 with surge. The initial phase of the study was performed to assess the optimum time to use the Pall leukocyte reduction filter #10 to produce leukoreduced platelets. In this study, single donor platelets were collected using the Haemonetics V50 at an ACD to blood ratio of 1:8 and the platelets were filtered immediately after collection, after storage at 22°C for 4 hours with rotation, and after storage at 22°C for 5 days with rotation.

The second phase of the study was done to analyze variables which could potentially affect platelets within the first 24 hours of storage at 22°C prior to cryopreservation, the time required to complete the testing of the platelets for the

current FDA-mandated infectious disease markers. The current policy is to freeze only single donor platelets obtained by plateletpheresis procedure and not pooled platelets obtained from multiple units of whole blood. Platelets were obtained from healthy donors by a plateletpheresis procedure using the Haemonetics V50 with surge. The following variables were evaluated: ACD to blood collection ratios of 1:8, 1:10, and 1:12; storage at 22°C for 4 hours with and without rotation followed by 20 hours of rotation for a total period of 24 hours; filtration of platelets stored at 22°C for 24 hours with rotation; cryopreservation of the platelets liquid stored for 24 hours and filtered or not filtered prior to freezing. In this phase 2 study, the quality of the platelets was assessed as described above.

METHODS

Plateletpheresis Procedure: Fourteen plateletpheresis products were collected at the Naval Blood Research Laboratory from volunteers meeting the requirements of AABB for healthy blood donors. None of the participants had taken medication for ten days prior to donation. The initial study was done to assess the effect of platelet leukoreduction using a Pall leukoreduction filter (LRF). In these 14 initial filtration studies, single donor platelets were collected into anticoagulant-citrate-dextrose-solution (ACD formula A, Baxter Healthcare Corp., Fenwal Division, Deerfield, IL) at an ACD:blood ratio of 1:8 using the Haemonetics V50 (Haemonetics, Braintree, MA), a discontinuous flow centrifugation system with the surge protocol. The single donor platelets were leukoreduced by filtering through a Pall LRF #10 (Pall Corp., Port Washington, NY). This was performed within 30 minutes of collection ("immediately"), 4 hours or 5 days after collection. Fourteen plateletpheresis products were split equally into two CLX bags. In seven studies one bag was filtered immediately, and the other filtered after 4 hrs rotation at 22°C on an Eberbach shaker at 140 oscillation per minute. In the remaining 7 plateletpheresis products half the product was filtered immediately and the remaining half stored at 22°C for five days on an Eberbach shaker at 140 oscillations per minute, and then filtered. Plateletpheresis product sterility was tested using supplemented peptone broth (Becton Dickinson, San Jose, CA, catalog #4955) culture. Plateletpheresis product volumes were determined by weighing the

plateletpheresis product, subtracting the weight of the bag and dividing this value by the specific gravity of platelet rich plasma. Platelet morphology was assessed by microscopy using the Moroff method[1]. Measurements of mean platelet volume (MPV), aggregation and thromboxane B_2 production in response to the addition of a combination of arachidonic acid and ADP, recovery from hypotonic stress, platelet and white cell counts were done before and after filtration. In addition, the plasma was assayed for thromboxane B_2, β-thromboglobulin, complement C3a, pH, PCO_2, and PO_2.

The second phase of this study addressed the effects of citrate concentration and plateletpheresis product non-rotation over the first 4 hours at room temperature after collection followed by 20 hours of rotation vs. rotation for the entire 24 hour period at 22°C. Prior to plateletpheresis, samples of peripheral blood were drawn from the donor in sodium citrate anticoagulant. Platelets were collected on three separate occasions from each of 6 normal donors into ACD formula A, using the Haemonetics V50 with the surge protocol. The donations were randomized into three groups with a four week rest in between with collections at a 1:8 ACD to blood ratio; a 1:10 ACD to blood ratio; and a 1:12 ACD to blood ratio. The plateletpheresis technician was the only individual aware of the collection ratio, and the code was not broken until the termination of the study for data analyses. Eighteen plateletpheresis procedures were performed, 6 at each collection ratio. In 15 plateletpheresis products an average of 295 ± 57 mL (mean ± SD) of platelets in plasma was collected with a total platelet count of $4.28 \pm 1.03 \times 10^{11}$. The products were sampled and a volume

of 140 ± 28 mL was put into each of two 600 ml PVC transfer packs (Baxter, Deerfield, IL, Cat # 4R2023). One bag was placed on an Eberbach shaker at 140 oscillations per minute for four hours at 22°C and the other left stationary at room temperature for four hours. Both bags were sampled at four hours, and both placed at 22°C on the rotator for 20 hours at which time the bags were again sampled. Those plateletpheresis products rotated at 22°C for the entire 24 hours were then filtered though a Pall LRF #10 prior to cryopreservation. The plateletpheresis products left stationary for the first 4 hours at room temperature then rotated at 22°C for 20 hours were not filtered prior to cryopreservation.

All samples collected were assayed for platelet count (Coulter Model JT), white count, microaggregates (Coulter Model TA II), mean platelet volume (Coulter Zf C1000), citrate level using the Philips Dual-Beam Spectrophotometer (PU8800 UV/VIS) , plasma thromboxane B_2 concentration (New England Nuclear Radioimmunoassay,) and, as determined by flow cytometry: a) platelet aggregate formation, b) the number of platelet-derived microparticles, c) the number of activated platelets (determined by platelet surface P-selectin), d) platelet surface GPIb, and e) platelet reactivity *in vitro* (determined by up-regulation of platelet surface P-selectin and down-regulation of platelet surface GPIb).

Following is an overview key of sampling time points and conditions for this second phase of this study provided to clarify the abbreviations used in the text, tables and figures:

OVERVIEW OF SAMPLING TIME POINTS:

Abbreviation	Description
PB	peripheral blood
0 h	0 hour plateletpheresis product at room temperature.
4 h (r)	4 hour plateletpheresis product (rotated) at 22°C.
4 h (nr)	4 hour plateletpheresis product (non-rotated) at room temperature.
24 h (r)	24 hour plateletpheresis product (rotated) at 22°C and then filtered prior to cryopreservation.
24 h (nr→r)	24 hour plateletpheresis product (non-rotated for the first 4 hours at room temperature and then rotated for 20 hours at 22°C) and not filtered prior to cryopreservation.

Platelet Cryopreservation: The 16 units of single donor 24-hour stored platelets which had been rotated at 22°C for the entire 24 hours were filtered through a Pall leukoreduction filter #10 and then frozen. Those products which were not rotated at room temperature for the first 4 hours only and then rotated for 20 hours at 22°C were frozen without filtration. The procedure for platelet cryopreservation has been previously described.[6] In each case, the products were transferred into a 1000 mL polyvinylcloride platelet freezing bag (Fenwal 4R2986). A volume of 50 mL of 27% dimethyl sulfoxide (DMSO) in saline was added at room temperature over a five minute period to achieve a final concentration of 6% DMSO. The platelets were

placed in an aluminum container and placed in a chest type freezer at -80°C to achieve a freezing rate of 2-3°C per minute and stored for a mean of 58 ± 8.9 (mean ± SD) days. The platelets were thawed in a 42°C water bath and diluted with a 250 mL volume of 0.9% NaCl, 0.2% glucose, 40 mg% inorganic phosphorus, pH 5 (Cytosol Laboratories, Braintree, Ma). The platelets were concentrated by centrifugation at 4500 x G for 5 minutes, the supernatant removed eliminating 95% of the DMSO, and the platelets resuspended in 50 mL of thawed previously-frozen autologous ACD plasma. Samples of the thawed, washed platelets were assayed for platelet count, white cell count, and mean platelet volume and microaggregates (Coulter Model TA II).

Microaggregate Analysis by the Electronic Impedance Method: Microaggregates in the fresh, 24 hour liquid stored, and filtered and non-filtered plateletpheresis units were measured electronically in a Coulter Model TA-II counter (Coulter Electronics, Inc. Hialeah, Fl). The number and size of the particles suspended in a conductive liquid were assigned to one of the 16 successive channels according to their size. The TA II analyzer counts the number of particles in each of the channels. The analyzer performs this by forcing the suspension to flow through a small aperture. As the particle passes through the aperture, it changes the resistance between the electrodes. This produces a current pulse of short duration, having a magnitude proportional to the size of the particle. Data are reported as the percentage of the total particles in channels 1-6 and the percentage of the total particles located in

channels 7-16. Platelets are counted in channels 1 to 6 and platelet aggregates are counted in channels 7-16 which measures particles of 11 microns or greater in size.

Filtration of Plateletpheresis Products using the Pall Filter: The Pall Leukocyte Reduction Filter (LRF) #10 was used according to the manufacturers instructions (Pall Corp., Port Washington, NY). After white cell and platelet counts are made and plateletpheresis product volumes were measured, the following calculations were made:

$$\% \text{ White cell Removal} = 100 - \frac{\text{post filtration white count} \times \text{post filtration volume}}{\text{pre filtration white count} \times \text{pre filtration volume}}$$

and

$$\% \text{ Platelet Loss} = 100 - \frac{\text{post filtration platelet count} \times \text{post filtration volume}}{\text{pre filtration platelet count} \times \text{pre filtration volume}}$$

Platelet Recovery from Hypotonic Stress: Platelet counts were measured using an automated particle counter (Coulter model JT). The count was adjusted to 5×10^8 /mL with autologous platelet poor plasma. A Philips PU 8800 UV/VIS

spectrophotometer measuring light transmittance at 610 nm was used for these measurements. The spectrophotometer was blanked to a sample of 300 µl each of platelet-poor-plasma and 0.9% NaCl. Light transmission was monitored for 10 minutes on samples of 300 µl adjusted plateletpheresis product/300 µl 0.9% NaCl (control) and 300 µl adjusted plateletpheresis product/300 distilled H_2O (test). The sample light transmittance was monitored continuously for 10 minutes and the results calculated as follows:

$$\% \text{ Recovery from Hypotonic stress} = \frac{\text{Secondary decrease in transmittance for 10 minutes}}{\text{Initial increase in transmittance}}$$

White Cell Counts. This assay utilizes propidium iodide fluorescent DNA stain, a fluorescent microscope and a large-volume (50 mm^3) Nageotte chamber for accurate counting of low-levels of white cells. Equal volumes of plateletpheresis product and propidium iodide solution (propidium iodide 200 µg/mL, sodium citrate 100 µg/mL and NP-40 0.32% in distilled H_2O) were incubated in the dark for 5 minutes. A 200 µl volume of this was loaded onto the Nageotte chamber and the cells were allowed to settle for 5 minutes. The brightly illuminated nucleated white cells were counted in the entire counting field (50 mm^3) using a Zeiss fluorescent microscope equipped with a tungsten lamp UV light source. The resulting count was multiplied times 40 to calculate the white cell number/mL. This was multiplied

by the measured volume (mL) of the plateletpheresis product to obtain the total white cell count.

Plasma β-thromboglobulin Assay: The platelet-specific protein β-thromboglobulin (β-TG) was measured using a commercially available radioimmunoassay (Code # IM.88, Amersham Corporation, Arlington Heights, IL) and used according to the manufacturers instructions. The presence of plasma β-TG in the plateletpheresis product is an indicator of the platelet release reaction.[7] The results are derived from a standard curve and are expressed as ng/mL.

Human complement C3a Assay: Human complement C3a was measured using a commercially available radioimmunoassay (Code # RPA518, Amersham Corporation, Arlington Heights, IL) and used according to the manufacturers instructions. Samples of cell-free plateletpheresis product plasma was prepared by centrifugation for 10 minutes at 1650 x G and kept frozen at -80°C until assayed. The presence of complement C3a is an indicator of the complement activation. The results are derived from a standard curve and are expressed as ng/mL.

Platelet Aggregation in Response to 0.5 mg/mL Arachidonic Acid and 0.2 μM ADP: Platelet aggregation was measured at 37°C using the Biodata aggregometer and Biodata reagents (Biodata Corp., Hatboro, PA). The plateletpheresis product was adjusted to a platelet count of approximately 5.0 x 10^8/ml. A 50 μl combination of 5

mg/mL arachadonic acid and 2 µM adenosine diphosphate (ADP) was added to 450 µl of diluted platelets (0.5 mg/mL arachadonic acid and 0.2 µM ADP final concentrations). The platelet aggregation pattern was recorded for 5 minutes. The 5 minute aggregation patterns were analyzed by digitizing the area under the curve using the Kurta Is/ONE input system and the data reported as digitizer units for the five minute period of aggregation.

Plasma pH, PCO_2 and PO_2 Assay: The plasma pH, PCO_2, and PO_2 were measured at 37°C on the IL Blood Gas Manager 1312 (Instrumentation Laboratories, Lexington, MA) in non-diluted samples drawn into a tuberculin syringe, capped tightly. Samples were run within 5 minutes, or kept on wet ice for up to 2 hours.

Mean Platelet Volume Measurement: The mean platelet volume of the fresh and liquid stored platelet concentrates was measured using the Coulter ZF/C1000 System (Coulter Electronics, Hialeah, Fl.). As platelets suspended in a solution pass through an aperture they displace an equal volume of electrolyte solution and produce a resistance in the path, the magnitude of which is directly proportional to the particle size. The instrumentation comprises of three main units, The Model Zf, the C1000 channelizer, and the computer interface. The model Zf counts and sizes all particles and transfers the information to the channelizer which assigns particles to channels based on size. This information is transferred to the computer interface, where the Accucomp program calculates the mean particle/platelet

volume. The mean platelet volume is reported in μm^3 and is the average of the volumetric size of the platelets in the sample.

Citrate Level Measurement: The level of citrate in the plateletpheresis product was measured with the Citric Acid Mannheim Boeringer Kit (UV method) on the Philips PU8800 UV/VIS Spectrophotometer. Samples were centrifuged in a Sorvall Model RT-7 at 280 x G for ten minutes and the cell free plasma removed and frozen at -20°C until assayed. Results are reported in grams per liter (g/L).

Thromboxane B_2 Assay: Thromboxane B_2 (a stable breakdown product of thromboxane A_2) was measured using a commercially available radioimmunoassay (New England Nuclear, Boston, MA) and used according to the manufacturers instructions. The presence of thromboxane B_2 in the plasma of plateletpheresis products is an indicator of platelet thromboxane synthase activity which occurs with platelet activation . In this study the level of thromboxane in the fresh and 24 hour stored plateletpheresis products were measured in the cell free plasma, prepared by centrifuging the sample at 1650 x G and 4°C for 10 minutes. The cell free plasma samples were frozen at -80°C until assayed.

In addition, platelet production of thromboxane B_2 per platelet was measured in the supernatant after aggregation with a combination of 0.5 mg/mL arachadonic acid and 0.2 µM ADP. After 5 minutes of aggregation, ibuprofen (0.02 ng/mL final concentration) was added to the cuvette to arrest thromboxane production and

placed on ice. The sample was then centrifuged (1650 x G, 4°C) and the plasma frozen at -80°C until assayed as described above. An unaggregated sample was treated identically to establish baseline thromboxane B_2 data. Platelet counts were performed on samples of the initial PRP used for aggregation and adjusted for dilution by the agonist and ibuprofen additions. The thromboxane production per platelet was calculated as follows:

$$\text{Thromboxane production per platelet} = 10 \times \frac{\text{Aggregated } TXB_2 \text{ Value - Baseline } TXB_2 \text{ value}}{\text{Platelet Count/mL} \times 10^8}$$

Preparation for flow cytometry: These methods are essentially as previously described for whole blood flow cytometry.[2,5,8] There were no centrifugation, gel filtration, vortexing, or stirring steps that could artefactually activate platelets. Peripheral blood and plateletpheresis product samples were incubated (22°C, 15 minutes) with the following agonists: thrombin 2 U/mL (gift of Dr. John Fenton) with 2.5 mM gly-pro-arg-pro (GPRP, an inhibitor of fibrin polymerization purchased from Calbiochem, La Jolla, CA), a combination of ADP 10 µM and arachidonic acid 50 µg/mL (both purchased from Biodata, Hatboro, PA), or modified HEPES-Tyrodes (H.T.) buffer only (137 mM NaCl, 2.8 mM KCl , 1 mM $MgCl_2.6H_2O$, 12 mM $NaHCO_3$, 0.4 mM Na_2HPO_4, 5.5 mM Glucose, 10 mM HEPES and 0.35% bovine serum albumin, all buffer components were purchased from Sigma, St. Louis, MO) Samples were then fixed by adding 1%

formaldehyde (final concentration, ultrapure methanol-free formaldehyde purchased from Polysciences, Warrington, PA) for 30 minutes at 22°C, diluted 10 fold with H.T. buffer and stored at 4°C until labeled with monoclonal antibodies for flow cytometry. Aliquots of this preparation were incubated with anti-GPIIIa-FITC (DAKO, Carpinteria, CA) at a near saturating concentration and biotin conjugates (biotinylated in our lab using biotin-X-NHS purchased from Calbiochem) of either, S12 (gift of Dr. R. McEver), 6D1 (gift of Dr. B. Coller) or the equivalent concentration of isotypic control murine IgG_1 (Sigma) at a saturating concentration for 15 minutes at 22°C. This was followed by adding streptavidin-R-phycoerythrin (Jackson Immunoresearch, West Grove, PA) for an additional 15 minutes at 22°C. Samples were then diluted 10 fold and run on a Coulter Epics PROFILE flow cytometer. DNA check and standard bright flow cytometry beads purchased from Coulter (Epics division, Hialeah, FL) were used for daily instrument calibration. Appropriate color compensation was set for fluorescence 1 (FL1, FITC) and fluorescence 2 (FL2, R-phycoerythrin) using 525 and 575 bandpass filters respectively. All data was saved in flow cytometry histogram files and analyzed using Coulter ELITE software version 2.21. Platelets were identified based on binding of the anti-GPIIIa-FITC antibody (FL1). Biotinylated test antibody binding expressed as linear mean fluorescence was collected based on R-phycoerythrin fluorescence (FL2). Percent of control fluorescence data was normalized to the daily peripheral blood sample after treatment with thrombin for S12 and after treatment with buffer only for 6D1.

Platelet aggregates and microparticles were determined by flow cytometry, using the log forward light scatter (LFS) parameter as a measure of platelet size. The log orthogonal light scatter signal shown in some figures is affected by changes in cell size, but it is influenced more by the shape and granularity of the cell. Only those particles that bound the GPIIIa-specific antibody (platelet-specific) were analyzed. Three methods were used to calculate the % aggregates in the sample: Method 1: Percent positive LFS events using the peripheral blood sample as the control; Method 2: Overton subtraction of the peripheral blood control LFS histogram from the test histogram using Coulter Cytologic software version 2.1; Method 3: Mean linear forward light scatter value where the peripheral blood control was assigned 100 units.

Platelet-derived microparticles were also determined by flow cytometry, using log forward light scatter as a measure of platelet size. For analysis of the number of platelet-derived microparticles by log forward light scatter, the control peripheral blood sample region was set at 4% (at the base of the distribution) of all platelet-specific events (positive for GPIIIa). While 1% regions are typically used in % positives analysis, this percentage is too small for % negative-type analysis as incidental sample "noise" can easily account for an additional 1-2% of events despite the use of the platelet-specific threshold (anti GPIIIa antibody). In addition, 4% is a conservative estimate of the number of platelet-derived microparticles[9,10] present in an unactivated platelet preparation and, as can be seen in figure 5, the plateletpheresis product samples did not differ significantly from the peripheral blood control sample. The 4% microparticle region typically included all counts up to channels 17-20 of the 64 channel

histogram (25-30% of the measurable range), large enough to allow inclusion of any microparticles formed during the 24 hour period under study. This analysis region was applied to all subsequent samples. All data are expressed as the mean ± S.E.M. with * indicating p-values of <0.05 (by paired or non-paired student's *t* test, Sigmaplot program, Jandel Scientific).

RESULTS

Although plateletpheresis procedures may produce a platelet concentrate which is very low in white cell content compared to manual preparation of platelets from whole blood, there are still significant numbers of white cells which may be removed by filtration. Table 1 reports the quality of the plateletpheresis product subjected to filtration by the Pall LRF#10 within the first 30 minutes after collection (immediate) or 4 hours after collection and storage at 22°C with rotation. There are no significant differences in volume loss, white cell removal or platelet recovery (Table 1).

Table 2 reports an approximately 16% reduction in total platelet count over 5 days of storage, with leukoreduction filtration reducing the platelet count on day 0, but not on day 5 of 22°C storage post collection. The total number of white cells was unchanged from days 0 to days 5, and leukoreduction filtration caused a 3.4 to 3.5 log removal of those cells from the plateletpheresis products on both days of 22°C storage.

Table 3 compares plateletpheresis products which were leukoreduced within 4 hours of storage at 22°C with rotation and 5 days post collection and storage at 22°C with rotation. Filtration had no significant effect on platelet morphology (Moroff method), mean platelet volume, thromboxane production or aggregation in response to a combination of ADP and arachadonic acid (ADP+AA) or recovery from hypotonic stress on day 0 post collection. Similarly, filtration on day 5 post collection had no effect on these measurements with the exception of a significantly reduced mean platelet volume. Although this suggests removal of larger platelets, it was not accompanied by a

reduction in platelet count after filtration (Table 2). Comparison of days 0 and 5 indicated a reduction in the platelet morphology score and a decreased platelet reactivity to the ADP+arachadonic acid addition both by extent of aggregation and production of thromboxane B_2. The recovery from hypotonic stress was similar on days 0 and 5 after plateletpheresis product collection and storage at 22°C with rotation (Table 3).

The pH and PCO_2 on day 0 showed no change with leukoreduction filtration while PO_2 increased slightly, but significantly (table 4, upper panel). Day 0 plasma thromboxane B_2, β-thromboglobulin, and complement C3a were reduced by filtration, this was significant in the case of the complement C3a. Leukoreduction filtration on day 5 post collection of 22°C storage resulted in a significantly reduced pH (table 4, lower panel). The PCO_2, which was reduced greater than 2 fold over the 5 days of storage was unchanged by filtration. The PO_2, which was level over the 5 days of storage was again increased somewhat by filtration, and the effect was significant. The thromboxane B_2, which increased only slightly over the 5 days of storage was unchanged by filtration. The β-thromboglobulin, which was considerably increased over the 5 days of storage, was reduced significantly by filtration. Complement C3a was significantly increased over the 5 days of storage, and was also significantly reduced by leukoreduction filtration (table 4).

Tables 5 through 8 report the effects of 5 day liquid storage at 22°C with rotation of plateletpheresis products which were either not filtered or leukoreduced using a Pall LRF#10 filter on the day of collection. Table 5 shows insignificant differences in the

platelet morphology score and mean platelet volume between the filtered and unfiltered products. Five day 22°C storage did result in a significant reduction in the morphology score in both the filtered and unfiltered groups, while the mean platelet volume did not change significantly.

Platelet aggregation in response to a combination of arachadonic acid and ADP was significantly reduced with time of 22°C storage in both the leukoreduced and unfiltered plateletpheresis products (table 6, top panel). While this aggregation was not significantly different between the filtered and unfiltered groups, the filtered platelets did consistently aggregate to a greater extent than the unfiltered platelets. Thromboxane B_2 production in these same samples (table 6, middle panel) was similar for both the filtered and unfiltered platelets. Time of 22°C storage did result in a decreased thromboxane B_2 response, but the differences were not significant. Platelet response to hypotonic stress remained virtually unchanged over the course of 22°C storage for both the unfiltered and leukoreduced plateletpheresis products (table 6, lower panel).

Leukoreduction filtration with the Pall LRF#10 resulted in reduced levels of plasma thromboxane B_2, β-thromboglobulin, and complement C3a (table 7). Only the C3a reduction was significant with 74% of the C3a being removed on day 0 of storage. Thromboxane B2 and β-thromboglobulin were reduced 28% and 39% respectively by filtration on day 0. Although plasma thromboxane B_2, β-thromboglobulin, and complement C3a all increased with time of 22°C storage, the filtered plateletpheresis product levels remained less than the unfiltered on each storage day.

Table 8 reports the plasma pH, PCO_2, and PO_2 over 5 days of 22°C storage of leukoreduced and unfiltered plateletpheresis products. Their were little differences between the filtered and unfiltered products. The pH increased significantly over time of storage, but did not rise to unacceptable levels in either the filtered or unfiltered groups. Plasma PCO_2 decreased significantly over time in storage in both the filtered or unfiltered groups. Plasma PO_2 remained nearly unchanged over time in storage in both the filtered or unfiltered groups, although filtration caused a temporary 20% increase on day 0.

Table 9 shows the results from a set of plateletpheresis products which were split and the 2 halves filtered sequentially through a single Pall leukoreduction filter #10. The products were prepared at an ACD:blood ratio of 1:8 on a Haemonetics V50 with surge. The platelets were stored at 22°C for 4 hours at 22°C with rotation prior to filtration. The differences between the product's first and second portions are indicative of filtration changes occurring during the course of leukoreduction filtration. The flow rate was reduced by 40% in the second portion. While the platelet recovery post-filtration did not change, the leukocyte removal improved during the filtration of the second portion.

The second phase of this report consists of a prospective, blinded, and randomized study addressing the effects of ACD concentration and rotation vs. no rotation for the first 4 hours of 24 hour storage. Prior to plateletpheresis the estimated ACD:blood ratio was pre-set on the Haemonetics V50 instrument. The actual ACD:blood ratio was calculated from the volume of ACD used and the volume of blood

processed during the procedure. The actual initial ACD:blood ratio was slightly lower than the estimated ratios (Figure 1). The measured citrate levels (in g/L) in the liquid stored products at all sampling points also reflected the changes in the anticoagulant used during the plateletpheresis procedure and were significantly different from each other (Table 10). For clarification purposes here and throughout the results, tables and figures, continuously rotated samples are indicated by "(r)", non-rotated by "(nr)" and the 4 hour non-rotated for the first 4 hours then rotated for 20 hours by "(nr→r)". The non-rotated platelets were stored at room temperature and the rotated platelets were stored at 22°C.

Plateletpheresis often produces a product which contains some small platelet aggregates which are not apparent macroscopically. Common techniques such as observing the swirl of the plateletpheresis product are insufficient to detect these microaggregates. The platelet count would presumably increase as aggregates produced upon collection disaggregated. However, little evidence of this trend was observed in this study. The total platelet count measured by the Coulter cell counter model JT using the electronic impedance method (Table 11) was not significantly different between the three ACD:blood groups at any timepoint. However, in the 1:12 ACD:blood group, the platelet count at time 0 was less, and it increased with storage suggesting some disaggregation over time. The overall trend for the three ACD:blood groups would indicate a greater platelet yield would be expected using an ACD:blood ratio of 1:8 rather than ACD:blood ratios of 1:10 or 1:12. In addition, following leukoreduction filtration of the plateletpheresis products using the Pall

LRF#10 the percent platelet loss was minimal in the 1:8 ACD:blood group, while 20% was lost after filtering both the 1:10 and 1:12 ACD:blood groups (Table 11) further reducing the total platelet yield.

There was no significant difference in the white count between the three citrate level groups (Table 12). The 1:8 ACD:blood group did tend to have the least number of white cells, however, suggesting more efficient functioning of the plateletpheresis discontinuous flow centrifugation system under these conditions in these matched donor groups. The white blood cell reduction with filtration was significant in all three ACD:blood ratio groups (Table 12).

There was no significant difference in the mean platelet volume (Table 13) between the 3 ACD:blood ratio groups, however there are some indications of an aggregate effect. The mean platelet volume of the 1:10 and 1:12 ACD:blood ratio groups tended to be slightly higher and more variable than the 1:8 ACD:blood group (Table 13). Further implementation of impedance methodology yielded no significant difference in the TA II data (aggregate analysis) between groups in either the single platelet channels 1-6 or the aggregate channels 7-16 (Table 14). There was a reduction with filtration in the percent aggregates (channels 7-16), and a corresponding percent increase in the channel 1-6 single platelet group. When the products are filtered, there is a greater reduction in the total platelet count in the 1:10 and the 1:12 ACD:blood groups (Table 11) suggesting once again the presence of aggregates. This aggregate reduction with filtration is also seen in the TA II data on table 14, but the observation was similar for all three groups.

Plasma thromboxane B_2 was initially greater in the 1:10 and 1:12 ACD:blood groups as compared to the 1:8 ACD:blood group (Table 15). While the effect was not significant, it would indicate a relatively lower level of platelet activation during the collection process at a 1:8 ACD:blood ratio. This stimulatory effect which has caused activity of platelet thromboxane synthase appears to continue for the initial period following plateletpheresis product harvest. The 4 hour plasma thromboxane B_2 is considerably higher than the initial level (compare Table 15 columns 2 and 3) while the level is relatively stable thereafter. While there is no evidence of differences in the level of plasma thromboxane B_2 between the 4 hour rotated (r) and non-rotated (nr) samples, it seems logical to minimize agitation of the plateletpheresis product during production of thromboxane.

A major objective of this study was to establish ideal conditions pre-freeze for the 24 hour storage period. The post thaw-wash recovery data indicate no significant differences in the three ACD:blood ratios (Table 16). It is notable, however, that the range of recoveries is greater in the lower 2 ACD:blood groups with some points being actually greater than 100%. This could be disaggregation caused by the freeze-thaw process or production of platelet microparticles which fall within the size range of normal platelets and counted as such by the Coulter Model JT particle analyzer. The 1:12 ACD:blood group included the plateletpheresis products with the lowest recovery in this study. There were no significant differences between the white cell filtered and non-filtered groups with regards to the post thaw-wash recovery of platelets (Table 16).

Analysis of platelet aggregates in post thaw-wash cryopreserved products by impedance methodology yielded significant differences between the filtered and non-filtered groups (Table 17). After the thaw-wash procedure, the filtered platelets were >90% single platelets as defined by their falling in channels 1-6 on the TA-II particle analyzer regardless of ACD concentration. Non-filtered platelet preparations contained 7-11% more aggregates as defined by their falling in channels 7-16 on the TA-II particle analyzer, again regardless of ACD concentration. No significant differences were observed at the various ACD concentrations with regard to their aggregate content by this method.

Analysis of total white cell count in post thaw-wash cryopreserved products yielded significant differences between the filtered and non-filtered groups, as expected (Table 18). White cell concentration between the three groups was similar. A proportion of the white cells included in the pre-freeze product (see table 12) did not remain intact after the cryopreserved platelet product was thawed and washed.

While the electrical impedance methods yielded no significant differences between the ACD:blood ratio sets with regard to platelet size or aggregation, flow cytometric methods employing platelet-specific monoclonal antibodies for cellular identification were able to detect small aggregates in the plateletpheresis products. Three methods of quantifying these were employed.

Figure 2 illustrates the detection of up to 10% aggregates using a "percent positives" approach. The peripheral blood sample serves as the negative control,

where an analysis region is placed above the peak on a log forward light scatter (LFS) histogram. All subsequent samples employ this same analysis region, where platelet-specific particles with increased LFS can be detected. Using ACD:blood ratios of 1:10 or 1:12 resulted in a significant number of aggregates as compared to collection at 1:8 ACD:blood (figure 2). After 4 hours plateletpheresis products using ACD:blood ratios of 1:10 or 1:12 either rotated or non-rotated during this period disaggregated to levels insignificantly higher than the plateletpheresis product collected at 1:8 ACD:blood. However, plateletpheresis products collected at 1:8 ACD:blood tended to aggregate somewhat during this same period. After 24 hours all plateletpheresis products were less aggregated than at the 4 hour period, with only the plateletpheresis products collected at 1:10 ACD:blood showing significantly more aggregates than the 1:8 ACD:blood plateletpheresis product.

Figures 3 and 4 illustrate two alternative methods for detecting small platelet aggregates using the LFS parameter of flow cytometry data. The Overton subtraction method (figure 3) overlays the 2 histograms (test and peripheral blood control) subtracting events at all points independent of the analysis regions. This was considered a more sensitive approach to detecting small quantities of microaggregates since platelets are inherently variable in size (1 to 5 μm in diameter). For example, an aggregate of 2-3 platelets may well fall in the LFS (size) range of a large single platelet. Overton subtraction did result in greater numbers of detected aggregates (figure 3), but T-test results proved to be less significant. This was perhaps the result of greater variability in the data set due to the increased sensitivity of the method. Potential use of

this test on an individual plateletpheresis product basis may be best served by the Overton subtraction technique if peripheral blood samples are used as a control.

The flow cytometer provides relative linear mean data on the LFS parameter. This information was used as the basis of the third method employed to detect platelet aggregates (figure 4). While this is the least sensitive of the 3 methods, it requires no calculations after data acquisition, making it the most convenient. Similar patterns of aggregation and disaggregation can be observed, as seen with methods 1 and 2. It should be noted that the degree of aggregation observed in these plateletpheresis products would not in any way preclude there use in a clinical setting. Minimizing the formation of aggregates during collection however, is an important step toward reducing platelet activation and producing a better plateletpheresis product.

In addition to detection of the larger platelet derived aggregates, analysis of log forward light scatter (LFS) data allows detection of the smaller platelet-derived microparticles. No significant microparticle generation was observed during the 24 hour time course of this study (figure 5).

Figure 6 shows a complete set of flow cytometry light-scatter contour plots from one donor after collecting plateletpheresis products on 3 occasions using the 3 ACD:blood ratios. The 0 hour plateletpheresis product sample collected at 1:12 ACD:blood shows the typical light-scatter pattern of an aggregated preparation. Disaggregation after only 4 hours of storage (following the histograms further to the right) can be observed as the loss of the "tail" on the upper right of the contour plot.

Figure 7 (A and B) contains peripheral blood control and 0 hour plateletpheresis product log light-scatter plots for all 5 donors. Note the characteristic aggregate "tail" in the 1:10 and/or 1:12 ACD:blood ratio plateletpheresis products for every donor studied. If this type of pattern consistently persisted for more than 24 hours without disaggregating, the plateletpheresis product collection procedure would need to be re-evaluated.

Platelet surface GPIb (CD42b) is the glycoprotein receptor involved in initial adhesion to damaged subendothelium via von Willebrand factor.[11] Loss of this receptor would be expected to indicate loss of this specific function, resulting in an inferior transfusion product. However, surface GPIb levels were not significantly affected by the different ACD:blood ratios used in this study (figure 8, top panel). Significant alterations of the surface GPIb over the 24 hour time course of liquid preservation was not seen, as can be expected from longer term storage.[12,13] Surface GPIb downregulates after activation,[14] and no significant difference from the peripheral blood control was observed after the addition of either thrombin (2 Units/mL) or the combination of ADP (10 µM) and arachadonic acid (50 µg/mL) (figure 8, lower 2 panels).

P-selectin is an alpha granule membrane protein which is surface exposed upon activation (concurrent with degranulation).[15] Conditions which result in platelet aggregation may or may not result in degranulation. For example, the potent aggregating agent ADP is a weak degranulator when used alone. Despite the appearance of microaggregates in platelet plateletpheresis products prepared for this study, detectable platelet activation was minimal (figure 9). The low levels of

surface P-selectin observed on all plateletpheresis product samples in the absence of added agonist (figure 9, upper panel) indicate little platelet degranulation occurred as a result of the plateletpheresis procedure at any ACD:blood ratio. There was a slight loss of reactivity to thrombin or the combination of ADP and arachadonic acid in some of the plateletpheresis product samples (figure 9, lower 2 panels), but not significantly. The lowest ACD:blood ratio (1:12) tended to maintain reactivity better than other ACD:blood ratios used. This could be considered less optimal since platelets ideally should be kept in a quiescent state during storage. Reactivity to an added agonist may indicate a relative tendency to respond to a storage-induced activation stimulus.

DISCUSSION

Filters designed to remove white blood cells from whole blood, red blood cell concentrates, and platelet concentrates may entrap red blood cells, hemolyze the red blood cells, activate the white blood cells and release white blood cell products, remove platelets, activate the platelets and release platelet products, activate the complement system and produce bradykinin.[1,16-19] In this study, removal of white blood cells from single donor platelets obtained by plateletpheresis using the Haemonetics V50 with surge was studied. Filtration of the platelets was done immediately following collection and following storage at 22°C for as long as 5 days. The effects of filtration on the recovery of the platelets, removal of white blood cells, the in vitro quality of the platelets and the removal by filtration of components of complement and platelet products were studied.

While pre-storage filtration of platelet concentrates with leukocyte reduction filters can prevent some of the adverse effects of leukocyte metabolites produced during storage,[20,21] immediate filtration of the product after collection may not be ideal if the platelets are recovering from low level activation which may occur with the normal shear forces produced by an plateletpheresis procedure.[22] The time of filtration may affect the recovery and quality of the plateletpheresis product subjected to leukoreduction filtration.

The results from the initial study reported here show no significant differences in volume loss, white cell removal or platelet recovery in plateletpheresis products filtered

immediately or 4 hours post collection and storage at 22°C with rotation at an ACD:blood ratio of 1:8. In this case there was only a 1% increase in platelet recovery when the plateletpheresis product was filtered at 4 hours vs. immediately. However if any aggregates were present at time 0 and they were allowed to disaggregate, this difference could potentially be much greater.

Filtration itself had no significant effect on several platelet measurements including morphology (Moroff method), thromboxane production and aggregation response to ADP plus arachadonic acid, or recovery from hypotonic stress on either day 0 or 5 post collection. Mean platelet volume was significantly reduced on day 5 with filtration despite lack of a reduction in platelet count. Although removal of larger platelets by filtration seems the most logical explanation, changes in tonicity or charge with filtration may modulate the degree of cytoplasmic volume (i.e. swelling). In fact, comparison of days 0 and 5 indicated a reduction in the platelet morphology score, however, the recovery from hypotonic stress was only slightly reduced on day 5 after collection. Plasma measurements indicate some soluble factors are removed by leukoreduction filtration, some of which were significant on day 5 post collection. This consideration is supported by the fact that the negatively-charged polyester based Pall leukoreduction filter #10 utilizes chemical attraction to protonated compounds such as carboxylate and amino groups. A significant proportion of soluble β-thromboglobulin and complement C3a was removed by filtration. Reduction of soluble factors such as these are an important consideration with regard to minimizing any adverse side effects of leukodepleted platelet transfusion products.[23]

Plateletpheresis products which were either filtered using a Pall LRF#10 on the day of collection, or not filtered were examined for differing effects of 5 day liquid storage at 22°C with rotation. Platelet aggregation in response to a combination of arachadonic acid and ADP was significantly reduced with time of storage in both the leukoreduced and unfiltered products. The filtered platelets consistently aggregated to a slightly greater extent than the unfiltered platelets, suggesting improved platelet function after leukodepletion. However, thromboxane B_2 production in these same samples did not show a consistent improvement with leukoreduction. Leukoreduction filtration with the Pall LRF#10 again resulted in reduced levels of plasma thromboxane B_2, β-thromboglobulin, and complement C3a. The reduced level of these soluble factors remained throughout the 5 days of 22°C storage with rotation, indicating pre-storage filtration would still have a positive effect throughout the 22°C storage period.

In the second phase of this study, which addressed the collection conditions, preparation and storage during the first 24 hours post-collection, the platelet yield was greater in the 1:8 ACD:blood group as compared to the 1:10 and 1:12 groups, though not significantly by analysis of variance. Following leukoreduction filtration of the plateletpheresis products using the Pall LRF#10, the percent platelet loss was minimal in the 1:8 ACD:blood group as compared to both the 1:10 and 1:12 ACD:blood groups. It remains to be determined if the higher citrate level was somehow protective of the platelets pre-filtration. However, considering the increased initial yield and reduced platelet loss with leukoreduction filtration, the

most effective plateletpheresis harvest would be achieved using an ACD:blood ratio of 1:8.

The 4 hour plasma thromboxane B_2 (the stable breakdown product of thromboxane A_2, a potent platelet agonist) at all citrate concentrations is considerably higher than the initial levels suggesting active platelet thromboxane synthase during this period. The plasma thromboxane B_2 level is fairly stable after that point indicating relatively less enzyme activity during the latter period. While our data do not indicate differences between the rotated and non-rotated groups, it seems logical to minimize agitation of the plateletpheresis product during production of this platelet stimulant to avoid irreversible aggregation from occurring.

Establishing ideal conditions during the initial 24 hour period post-collection for obtaining an improved cryopreserved platelet product is needed. The various citrate levels studied indicate no apparent differences in post thaw/wash recovery. The fact that the range of recoveries was greater in the 1:10 and 1:12 ACD:blood groups indicates that 1:8 ACD:blood will yield a more consistent cryopreserved single donor platelet product. The 1:12 ACD:blood group included the plateletpheresis products with the lowest recovery.

Analysis of platelet aggregates in these thawed platelet products by the TA-II indicated the platelets filtered prior to freezing were more likely to remain as single platelets compared to the non-filtered platelet preparations. The non-filtered platelets contained about 10% more aggregates which may reflect an activation

process which involves intact or lysed white cells. Citrate concentration did not appear to have a role in the formation of these aggregates.

Current methods employed in the blood bank to asses plateletpheresis product quality include visual examination of swirl, pH measurement and possibly recovery from hypotonic stress for generalized membrane integrity confirmation. Although light scattering has been employed to assess the general morphology of platelet concentrates,[24,25] microscopy is the only option for analysis of platelet microaggregates and this is difficult, time consuming and highly subjective due to the small size of these cells. Automated methodology should be developed to quickly provide objective structural information to aid in the evaluation of the quality of plateletpheresis products. The increasing availability of flow cytometers for clinical use, and the rapid multiple-parameter capabilities of these instruments are ideally suited to provide this type of automated evaluation of transfusion products.

In addition to structural information and basic membrane integrity, the flow cytometer can evaluate critical cytoskelatal and platelet surface receptor functions which play a central role in hemostasis. The platelet surface contains binding sites for sub-endothelial matrix molecules that promote cell adhesion, agonist receptors that are linked via G proteins to phospholipases and other stimulus-response elements, and the GPIIb-IIIa (CD41/61, integrin $\alpha_{IIb}\beta_3$) complex which mediates platelet aggregation by providing an activation-induced receptor for fibrinogen and von Willebrand factor.[26] Activated platelets release and bind thrombospondin and express the alpha-granule membrane protein P-selectin (CD62P, GMP-140,

PADGEM protein), both of which are involved in the interaction of platelets with other circulating cells.[27,28] Activated platelets also provide a catalytic surface for procoagulant reactions that lead to thrombin generation. The specificity of monoclonal antibodies and the sensitivity of flow cytometry can be used to advantage to characterize these surface events during platelet storage and activation.[4]

By flow cytometry, we have demonstrated that an ACD:blood collection ratio of 1:8 resulted in a minimal number of platelet aggregates immediately after the preparation of platelet concentrates, whereas ratios of 1:10 or 1:12 resulted in more aggregates. Within 4 hours of storage there were no significant differences in the number of platelet aggregates, irrespective of the original ACD:blood ratio and of whether or not there was rotation. After 24 hours of storage of rotated platelet concentrates, there were no significant differences in the number of platelet aggregates, irrespective of the original ACD:blood ratio and whether or not there was rotation during the first 4 hours. In contrast, after 4 hours of non-rotation followed by 20 hours of rotation, the plateletpheresis products collected at an ACD:blood ratio of 1:8 resulted in a minimal number of platelet aggregates, whereas ratios of 1:10 or 1:12 resulted in significantly more aggregates. Platelet counts or mean platelet volume measurements are a less sensitive method of detection of platelet aggregates in platelet concentrates. Irrespective of the original ACD:blood ratio and of whether or not there was initial rotation of the platelet concentrates during the first 4 hours, no significant differences were detected in the number of platelet-derived microparticles during the first 24 hours

of storage at 22°C. Irrespective of the original ACD:blood ratio and of whether or not there was rotation of the platelet concentrates, no differences were detected in the number of activated platelets or in platelet reactivity, as determined by platelet surface expression of P-selectin or GPIb during the first 24 hours of storage.

In conclusion, flow cytometry is a more sensitive method for detection of platelet aggregates in platelet concentrates compared to the impedance methods currently in widespread use. Platelet collection using the Haemonetics V50 with surge produces a product with minimal platelet aggregates under the following conditions: a) ACD:blood collection ratio of 1:8 rather than 1:10 or 1:12, b) no rotation during the first 4 hours of storage, c) filtration after 4 hours in a stationary position.

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Table 1

Effect of Leukoreduction Filtration of Plateletpheresis Products on Volume Loss, Platelet Recovery, and White Cell Removal When Platelets are Filtered Through a Pall LRF#10 Immediately or 4 Hours after Collection at a 1:8 ACD to Blood Ratio (Mean ± SD, n=7)

Product Volume and Flow Rate

Time of Filtration	Pre-Filtration Volume (mL)	Post-Filtration Volume (mL)	Volume Loss (%)	Flow Rate (mL/min)
Immediate	**157 ± 63**	**150 ± 64**	**5.6 ± 3.2**	**52 ± 19**
4 Hours	**203 ± 62**	**192 ± 64**	**5.2 ± 2.0**	**51 ± 7**
ANOVA	**NS**	**NS**	**NS**	**NS**

Platelet Recovery

Time of Filtration	Pre-Filtration Platelet Count x 10^{11}	Post-Filtration Platelet Count x 10^{11}	Platelet Recovery (%)
Immediate	**2.11 ± 0.84**	**1.92 ± 0.84**	**89 ± 9**
4 Hours	**2.88 ± 0.68**	**2.58 ± 0.72**	**90 ± 3**
ANOVA	**NS**	**NS**	**NS**

White Cell Removal

Time of Filtration	Pre-Filtration White Cell Count x 10^{8}	Post-Filtration White Cell Count x 10^{5}	White Cell Log Reduction
Immediate	**7.7 ± 6.3**	**1.25 ± 2.17**	**3.84**
4 Hours	**7.7 ± 7.1**	**0.93 ± 0.9**	**3.88**
ANOVA	**NS**	**NS**	**NS**

Table 2

Effect of Filtration of Plateletpheresis Products through a Pall LRF#10 on Platelet and White cell Counts Performed on Day 0 and Day 5 of Liquid Storage at 22°C (Mean ± SD)

Total Platelet Count (n=6)

Time of Filtration	Pre-Filtration Platelet Count ($x 10^{11}$)	Post-Filtration Platelet Count ($x 10^{11}$)	ANOVA	Platelet Recovery (%)
Immediate	**1.9 ± 0.4**	**1.78 ± 0.45**	**NS**	**94%**
5 Days	**1.6 ± 0.3**	**1.62 ± 0.5**	**NS**	**100%**
AVOVA	**NS**	**NS**		

Total White Cell Count (n=5)

Time of Filtration	Pre-Filtration White Cell Count ($x 10^{5}$)	Post-Filtration White Cell Count ($x 10^{5}$)	ANOVA	White Cell Log Reduction
Immediate	**4200 ± 3400**	**2.2 ± 2.7**	**<0.0001**	**3.5**
5 Days	**4500 ± 3440**	**2.8 ± 6.6**	**<0.0001**	**3.4**
AVOVA	**NS**	**NS**		

Table 3

Effect of Filtration On Days 0 and 5 of 22°C Storage Using the Pall LRF#10 on Plateletpheresis Products Collected with a Haemonetics V50 with Surge at a 1:8 ACD to Blood Ratio on Platelet Measurements

Filtration on Day 0 (Immediate and 4 hour combined, Mean ± SD, n=6)

White cell Filtration Status	Platelet Morphology Score	TXB_2 Production per PLT ng x 10^{-6} (ADP+AA)	MPV (μm^3)	Aggregation Digitized Units/ 5 min (ADP+AA)	Percent Recovery from Hypotonic Stress
Pre-Filtration	**346 ± 8**	**590 ± 280**	**7.3 ± 1.0**	**238 ± 55**	**88 ± 104**
Post-Filtration	**343 ± 9**	**710 ± 480**	**7.3 ± 1.0**	**278 ± 31**	**92 ± 13**
ANOVA	**NS**	**NS**	**NS**	**NS**	**NS**

Filtration on Day 5 (Mean ± SD, n=5)

White cell Filtration Status	Platelet Morphology Score	TXB_2 Production per PLT ng x 10^{-6} (ADP+AA)	MPV (μm^3)	Aggregation Digitized Units/ 5 min (ADP+AA)	Percent Recovery from Hypotonic Stress
Pre-Filtration	**295 ± 4**	**236 ± 80**	**7.3 ± 0.8**	**118 ± 65**	**81 ± 3**
Post-Filtration	**294 ± 7**	**248 ± 120**	**6.8 ± 0.6**	**118 ± 71**	**78 ± 10**
ANOVA	**NS**	**NS**	**<0.05**	**NS**	**NS**

Table 4

Effect of Filtration On Days 0 and 5 of 22°C Storage Using the Pall LRF#10 on Plateletpheresis Products Collected with a Haemonetics V50 with Surge at a 1:8 ACD to Blood Ratio on Plasma Measurements

Filtration on Day 0 (Immediate and 4 hour combined, Mean ± SD, n=6)

White cell Filtration Status	pH	PCO_2	PO_2	TXB_2 (pg/0.1mL)	β-TG (ng/mL)	C3a (ng/mL)
Pre-Filtration	**6.99 ± 0.40**	**70 ± 7**	**100 ± 25**	**1020 ± 1200**	**5475* ± 5750**	**980 ± 577**
Post-Filtration	**6.98 ± 0.03**	**68 ± 5**	**120 ± 14**	**734 ± 250**	**2527 ± 3250**	**259 ± 93**
ANOVA	**NS**	**NS**	**<0.05**	**NS**	**NS**	**<0.05**

* n=4

Filtration on Day 5 (Mean ± SD, n=5)

White cell Filtration Status	pH	PCO_2	PO_2	TXB_2 (pg/0.1mL)	β-TG (ng/mL)	C3a (ng/mL)
Pre-Filtration	**7.06 ± 0.07**	**19.4 ± 4.0**	**116 ± 21**	**1979 ± 507**	**17854 ± 4408**	**10103 ± 3579**
Post-Filtration	**6.73 ± 0.25**	**25.2 ± 2.0**	**165 ± 19**	**1742 ± 1009**	**12560 ± 3781**	**4167 ± 1024**
ANOVA	**<0.05**	**NS**	**<0.001**	**NS**	**<0.01**	**<0.05**

Table 5

Effect of 22°C Storage on Pall LRF#10 Filtered vs. Unfiltered Platelet Concentrates Collected Using a Haemonetics V50 with Surge at a 1:8 ACD to Blood Ratio on Platelet Morphology Score and Mean Platelet Volume (Mean ± SD, n=6)

Platelet Morphology Score (Moroff method)

Filtration Status	Day 0 of Storage	Day 1 of Storage	Day 2 of Storage	Day 3 of Storage	Day 5 of Storage	ANOVA
Unfiltered	**346 ± 8**	**334 ± 10**	**310 ± 9**	**299 ± 5**	**293 ± 5**	**<0.001**
Filtered	**343 ± 9**	**329 ± 7**	**312 ± 10**	**296 ± 4**	**289 ± 4**	**<0.001**
ANOVA	**NS**	**NS**	**NS**	**NS**	**NS**	

Mean Platelet Volume (μm^3)

Filtration Status	Day 0 of Storage	Day 1 of Storage	Day 2 of Storage	Day 3 of Storage	Day 5 of Storage	ANOVA
Unfiltered	**7.33 ± 0.97**	**7.27 ± 0.89**	**7.10 ± 0.90**	**7.02 ± 0.76**	**7.35 ± 0.70**	**NS**
Filtered	**7.32 ± 1.00**	**7.00 ± 1.00**	**7.08 ± 0.97**	**6.98 ± 0.80**	**7.13 ± 0.75**	**NS**
ANOVA	**NS**	**<0.05**	**NS**	**NS**	**NS**	

Table 6

Effect of 22°C Storage on Pall LRF#10 Filtered vs. Unfiltered Platelet Concentrates Collected Using a Haemonetics V50 with Surge at a 1:8 ACD to Blood Ratio on Platelet Aggregation and Thromboxane B_2 Production in Response to 2 µM ADP plus 0.5 mg/mL Arachadonic Acid and Recovery from Hypotonic Stress (Mean ± SD, n=6)

Platelet Aggregation (5 minutes, digitized units)

Filtration Status	Day 0 of Storage	Day 1 of Storage	Day 2 of Storage	Day 3 of Storage	Day 5 of Storage	ANOVA
Unfiltered	**238 ± 55**	**198 ± 43**	**181 ± 30**	**171 ± 33**	**129 ± 64**	**<0.05**
Filtered	**278 ± 31**	**254 ± 19**	**206 ± 53**	**190 ± 29**	**149 ± 56**	**<0.001**
ANOVA	**NS**	**<0.05**	**NS**	**NS**	**NS**	

Platelet Thromboxane B_2 Production per Platelet (ng x 10^{-6})

Filtration Status	Day 0 of Storage	Day 1 of Storage	Day 2 of Storage	Day 3 of Storage	Day 5 of Storage	ANOVA
Unfiltered	**580 ± 280**	**700 ± 830**	**540 ± 380**	**500 ± 210**	**320 ± 210**	**NS**
Filtered	**710 ± 480**	**840 ± 760**	**380 ± 380**	**360 ± 160**	**320 ± 210**	**NS**
ANOVA	**NS**	**NS**	**NS**	**<0.01**	**NS**	

Percent Platelet Recovery from Hypotonic Stress

Filtration Status	Day 0 of Storage	Day 1 of Storage	Day 2 of Storage	Day 3 of Storage	Day 5 of Storage	ANOVA
Unfiltered	**88 ± 10**	**92 ± 8**	**86 ± 9**	**84 ± 6**	**82 ± 4**	**NS**
Filtered	**92 ± 13**	**92 ± 5**	**89 ± 7**	**89 ± 6**	**82 ± 5**	**NS**
ANOVA	**NS**	**NS**	**NS**	**<0.02**	**NS**	

Table 7

Effect of 22°C Storage on Pall LRF#10 Filtered vs. Unfiltered Platelet Concentrates Collected Using a Haemonetics V50 with Surge at a 1:8 ACD to Blood Ratio on Plasma Thromboxane B_2, β-Thromboglobulin, and Complement C3a (Mean ± SD, n=6)

Plasma Thomboxane B_2 (pg/0.1mL)

Filtration Status	Day 0 of Storage	Day 1 of Storage	Day 2 of Storage	Day 3 of Storage	Day 5 of Storage	ANOVA
Unfiltered	**1020 ± 1200**	**1070 ± 377**	**2007 ± 1765**	**1424 ± 406**	**2078 ± 513**	**NS**
Filtered	**734 ± 250**	**910 ± 594**	**1313 ± 565**	**1983 ± 702**	**1720 ± 690**	**<0.01**
ANOVA	**NS**	**NS**	**NS**	**NS**	**NS**	

Plasma β-Thromboglobulin (ng/mL)

Filtration Status	Day 0 of Storage	Day 1 of Storage	Day 2 of Storage	Day 3 of Storage	Day 5 of Storage	ANOVA
Unfiltered	**5475 ± 5751**	**9750 ± 8089**	**15895 ± 11206**	**16090 ± 8976**	**18545 ± 4291**	**NS**
Filtered	**3339* ± 3350**	**5083** ± 4100**	**7883* ± 5775**	**6763** ± 1095**	**14838 ± 5885**	**<0.01**
ANOVA	**NS**	**NS**	**NS**	**NS**	**NS**	

Plasma Complement C3a (ng/mL)

Filtration Status	Day 0 of Storage	Day 1 of Storage	Day 2 of Storage	Day 3 of Storage	Day 5 of Storage	ANOVA
Unfiltered	**980 ± 577**	**2683 ± 1349**	**4531 ± 2101**	**5894 ± 1915**	**12257 ± 6171**	**<0.001**
Filtered	**259 ± 93**	**1891 ± 949**	**2910 ± 1290**	**4600 ± 1755**	**8674 ± 5660**	**<0.001**
ANOVA	**<0.05**	**<0.02**	**<0.05**	**<0.02**	**NS**	

* n=3 for paired t-test
** n=4 for paired t-test

Table 8

Effect of 22°C Storage on Pall LRF#10 Filtered vs. Unfiltered Platelet Concentrates Collected Using a Haemonetics V50 with Surge at a 1:8 ACD to Blood Ratio on Plasma pH, PCO_2, and PO_2 (Mean ± SD, n=6)

Plasma pH

Filtration Status	Day 0 of Storage	Day 1 of Storage	Day 2 of Storage	Day 3 of Storage	Day 5 of Storage	ANOVA
Unfiltered	**6.99 ± 0.04**	**7.19 ± 0.06**	**7.21 ± 0.05**	**7.17 ± 0.04**	**7.03 ± 0.09**	**<0.001**
Filtered	**6.98 ± 0.31**	**7.17 ± 0.08**	**7.2 ± 0.72**	**7.18 ± 0.05**	**7.05 ± 0.10**	**<0.001**
ANOVA	**NS**	**NS**	**NS**	**NS**	**NS**	

Plasma PCO_2

Filtration Status	Day 0 of Storage	Day 1 of Storage	Day 2 of Storage	Day 3 of Storage	Day 5 of Storage	ANOVA
Unfiltered	**70 ± 7**	**37 ± 7**	**29 ± 5**	**24 ± 6**	**18 ± 5**	**<0.001**
Filtered	**68 ± 5**	**37 ± 8**	**30 ± 7**	**26 ± 6**	**21 ± 7**	**<0.001**
ANOVA	**NS**	**NS**	**NS**	**NS**	**NS**	

Plasma PO_2

Filtration Status	Day 0 of Storage	Day 1 of Storage	Day 2 of Storage	Day 3 of Storage	Day 5 of Storage	ANOVA
Unfiltered	**100 ± 25**	**94 ± 17**	**105 ± 18**	**107 ± 22**	**119 ± 21**	**NS**
Filtered	**120 ± 14**	**93 ± 23**	**97 ± 27**	**109 ± 25**	**114 ± 19**	**NS**
ANOVA	**<0.05**	**NS**	**NS**	**NS**	**NS**	

Table 9

Split Plateletpheresis Product Filtered through a Single Pall Leukoreduction Filter #10 (Mean ± SEM, N=4)

Stage of Filtration	Flow Rate (mL/min)	Pre-Filtration Total Platelet Count $\times 10^{11}$	Post-Filtration Total Platelet Count $\times 10^{11}$	Percent Platelet Recovery after Filtration	Pre-Filtration Total WBC Count $\times 10^{8}$	Post-Filtration Total WBC Count $\times 10^{5}$	White Cell Log Reduction
First Portion	**56 ± 11**	**-**	**1.68 ± 0.5**	**95 ± 7**	**-**	**33.6 ± 52.3**	**4**
Second Portion	**34 ± 14**	**-**	**1.37 ± 0.4**	**94* ± 4**	**-**	**21.5 ± 23.2**	**5**
Combined (Total)	**45 ± 14**	**3.22 ± 0.7**	**2.92 ± 0.9**	**89 ± 7**	**10.7 ± 8**	**55.1 ± 46.7**	**4.5**

* adjusted for volume loss.

Table 10

Measured Citrate Level (grams/liter, mean ± SD, n=5)
Fresh and Liquid Preserved Platelets

Ratio of ACD:blood	0 Hour Unit	4 Hour Rotated (r)	4 Hour non-rotated (nr)	24 Hour non-rotated for first 4Hrs and then rotated 20 Hrs (nr→r)	24 Hour rotated pre-filtration (r)	24 Hour Rotated and Filtered
1:8	4.11 ± 0.26	3.96 ± 0.30	4.30 ± 0.42	3.93 ± 0.59	3.76 ± 0.22	3.99 ± 0.15
1:10	3.24 ± 0.27	3.09 ± 0.21	3.16 ± 0.20	2.93 ± 0.39	3.10 ± 0.20	3.02 ± 0.47
1:12	2.83 ± 0.19	2.80 ± 0.29	2.73 ± 0.25	2.89 ± 0.21	2.86 ± 0.24	2.57 ± 0.40
ANOVA	.001	0.001	0.01	0.05	0.02	NS

Table 11

Total Platelet Count x 10^{11} (mean ± SD, n=5)
Fresh and Liquid Preserved Platelets

Ratio of ACD: blood	0 Hour Unit	4 Hour Rotated (r)	4 Hour non-rotated (nr)	24 Hour non-rotated for first 4Hrs and then rotated 20 Hrs (nr→r)	24 Hour Rotated pre-filtration (r)	24 Hour Rotated and then Filtered	% PLT Loss with Filtration
1:8	**1.61 ± 0.24**	**1.55 ± 0.48**	**1.54 ± 0.38**	**1.49 ± 0.25**	**1.56 ± 0.31**	**1.52± 0.51**	**3 %**
1:10	**1.55 ± 0.74**	**1.49 ± 0.67**	**1.45 ± 0.60**	**1.60 ± 0.71**	**1.55 ± 0.66**	**1.24± 0.40**	**20 %**
1:12	**1.28 ± 0.48**	**1.40 ± 0.55**	**1.48 ± 0.62**	**1.47 ± 0.58**	**1.45 ± 0.52**	**1.16 ± 0.45**	**20 %**
ANOVA	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	

Table12

Total White cell Count x 10^6 (mean ± SD, n=5)
Fresh and Liquid Preserved Platelets

Ratio of ACD: blood	0 Hour Unit	4 Hour Rotated (r)	4 Hour non-rotated (nr)	24 Hour non-rotated for first 4Hrs and then rotated 20 Hrs (nr→r)	24 Hour Rotated pre-filtration (r)	24 Hour Rotated and then Filtered	ANOVA pre-filtration to filtered
1:8	**273 ± 208**	**156 ± 102**	**225 ± 208**	**198 ± 157**	**276 ± 324**	**31 ± 48**	**<0.02**
1:10	**1399 ± 1225**	**764 ± 623**	**702 ± 469**	**524 ± 433**	**637 ± 596**	**16 ± 13**	**<0.02**
1:12	**474 ± 400**	**268 ± 169**	**537 ± 539**	**199 ± 123**	**162 ± 107**	**18 ± 28**	**<0.02**
ANOVA	**NS**	**<0.02**	**NS**	**NS**	**NS**	**NS**	

Table 13

Mean Platelet Volume (μm^3, mean ± SD, n=5)
Fresh and Liquid Preserved Platelets

Ratio of ACD:blood	0 Hour Unit	4 Hour Rotated (r)	4 Hour non-rotated (nr)	24 Hour non-rotated for first 4Hrs and then rotated 20 Hrs (nr→r)	24 Hour Rotated pre-filtration (r)	24 Hour Rotated and then Filtered
1:8	**7.42 ± 0.52**	**7.55 ± 0.67**	**7.44 ± 0.73**	**7.28 ± 0.63**	**7.24 ± 0.81**	**6.47 ± 0.17**
1:10	**8.30 ±1.20**	**8.40 ± 1.20**	**8.05 ± 1.55**	**7.80 ± 1.20**	**8.15 ± 1.05**	**7.80 ± 1.22**
1:12	**7.86 ± 0.82**	**7.54 ± 1.15**	**7.70 ± 0.92**	**7.88 ± 0.89**	**7.70 ± 0.85**	**7.04 ± 0.37**
ANOVA	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**

Table 14

Impedance Method Size Analysis using the Coulter TA II (mean ± SD, n=5) Fresh and Liquid Preserved Platelets

Platelets

Percent in channels 1-6 (3 to 10 microns)

Ratio of ACD:blood	0 Hour Unit	4 Hour Rotated (r)	4 Hour non-rotated (nr)	24 Hour non-rotated for first 4Hrs and then rotated 20 Hrs (nr→r)	24 Hour Rotated pre-filtration (r)	24 Hour Rotated and then Filtered	ANOVA pre-filtration to filtered
1:8	**92.14 ± 12.03**	**92.15 ± 12.06**	**92.27 ± 11.96**	**92.58 ± 11.42**	**92.45 ± 11.61**	**99.77 ± 0.13**	**<0.02**
1:10	**86.56 ± 12.61**	**79.13 ± 25.02**	**88.00 ± 11.45**	**88.21 ± 11.75**	**87.99 ± 11.33**	**99.35 ± 0.74**	**<0.02**
1:12	**94.64 ± 4.02**	**93.32 ± 5.92**	**93.79 ± 5.32**	**94.83 ± 4.86**	**94.93 ± 4.78**	**99.94 ±0.04**	**<0.02**
ANOVA	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	

Platelet Aggregates

Percent in channels 7-16 (11 to 101 microns)

Ratio of ACD:blood	0 Hour Unit	4 Hour Rotated (r)	4 Hour non-rotated (nr)	24 Hour non-rotated for first 4Hrs and then rotated 20 Hrs (nr→r)	24 Hour Rotated pre-filtration (r)	24 Hour Rotated and then Filtered	ANOVA pre-filtration to filtered
1:8	**7.86 ± 12.03**	**7.85 ± 12.06**	**7.73 ± 11.96**	**7.24 ±11.53**	**7.55 ± 11.61**	**0.23± 0.13**	**<0.02**
1:10	**13.44 ± 12.61**	**20.87 ± 25.02**	**11.99 ± 11.44**	**11.79 ± 11.75**	**12.27 ± 11.07**	**0.65± 0.74**	**<0.02**
1:12	**5.36 ±4.02**	**6.68 ± 5.92**	**6.21 ± 5.32**	**5.17 ± 4.86**	**5.07 ± 4.78**	**0.07± 0.04**	**<0.02**
ANOVA	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	

Table 15

Plasma Thromboxane B_2 (pg/0.1 mL, mean ± SD, n=5) Fresh and Liquid Preserved Platelets

Ratio of ACD:blood	0 Hour Unit	4 Hour Rotated (r)	4 Hour non-rotated (nr)	24 Hour non-rotated for first 4Hrs and then rotated 20 Hrs (nr→r)	24 Hour Rotated pre-filtration (r)	24 Hour Rotated and then Filtered
1:8	**122.65 ± 61.02**	**572.25 ± 482.65**	**605.70 ± 495.21**	**672.73 ± 829.47**	**1028.40 ± 698.12**	**ND**
1:10	**685.50 ± 836.88**	**1478.50 ± 1971.77**	**1630.00 ± 1978.42**	**1570.00 ± 1828.25**	**1636.75 ± 1913.08**	**694.00 ± 410.00**
1:12	**217.00 ± 72.29**	**650.50 ± 394.77**	**583.25 ± 285.18**	**885.25 ± 410.64**	**820.50 ± 345.10**	**913.00 ± 336.83**
ANOVA	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**

Table 16

Percent Freeze-Thaw-Wash Recovery (mean ± SD)

Ratio of ACD : blood	n=	Days Frozen	Filtered	Non-Filtered	ANOVA Filtered: Non-Filtered
1:8	**6**	**52 ± 37 Range = 30 - 120**	**78 ± 10 Range = 60 - 93**	**76 ± 8.5 Range = 62 - 79**	**NS**
1:10	**6**	**55 ± 51 Range = 30 - 210**	**82 ± 13 Range = 66 - 99**	**86 ± 20 Range = 61 - 112**	**NS**
1:12	**4**	**90 ± 60 Range = 30 - 180**	**76 ± 18 Range = 47 - 92**	**80 ± 25 Range = 47 - 117**	**NS**
ANOVA		NS	NS	NS	

Table 17

Impedance Method Size Analysis (Coulter TA II)
(mean ± SD)
Previously Frozen Platelets

Platelets
percent in Channels 1-6

Ratio of ACD : blood	n=	Filtered	Non-Filtered	ANOVA Filtered: Non-Filtered
1:8	**6**	**95.3 ± 1.1**	**85.4 ± 4.4**	**<0.0001**
1:10	**6**	**97.0 ± 2.3**	**90.0 ± 3.6**	**<0.0001**
1:12	**4**	**92.6 ± 7.5**	**83.9 ± 6.7**	**<0.0001**
ANOVA		**NS**	**NS**	

Platelet Aggregates
percent in Channels 7-16

Ratio of ACD : blood	n=	Filtered	Non-Filtered	ANOVA Filtered: Non-Filtered
1:8	**6**	**4.0 ± 1.6**	**15.0 ± 4.4**	**<0.0001**
1:10	**6**	**3.0 ± 2.3**	**10.0 ± 3.6**	**<0.0001**
1:12	**4**	**8.0 ± 7.0**	**16.3 ± 6.0**	**<0.0001**
ANOVA		**NS**	**NS**	

Table 18

Total White cell Count x 10^6 (mean ± SD)
Previously Frozen Platelets

Ratio of ACD : blood	n=	Filtered	Non-Filtered	ANOVA Filtered: Non-Filtered
1:8	**6**	**17 ± 10**	**89 ± 6**	**<0.05**
1:10	**6**	**19 ± 14**	**148 ± 6**	**<0.05**
1:12	**4**	**28 ± 16**	**209 ± 6.**	**<0.05**
ANOVA		**NS**	**NS**	

Figure 1

Comparison of the estimated and actual ACD:blood collection ratio for platelet concentrates. Data are mean ± S.E.M., n = 5.

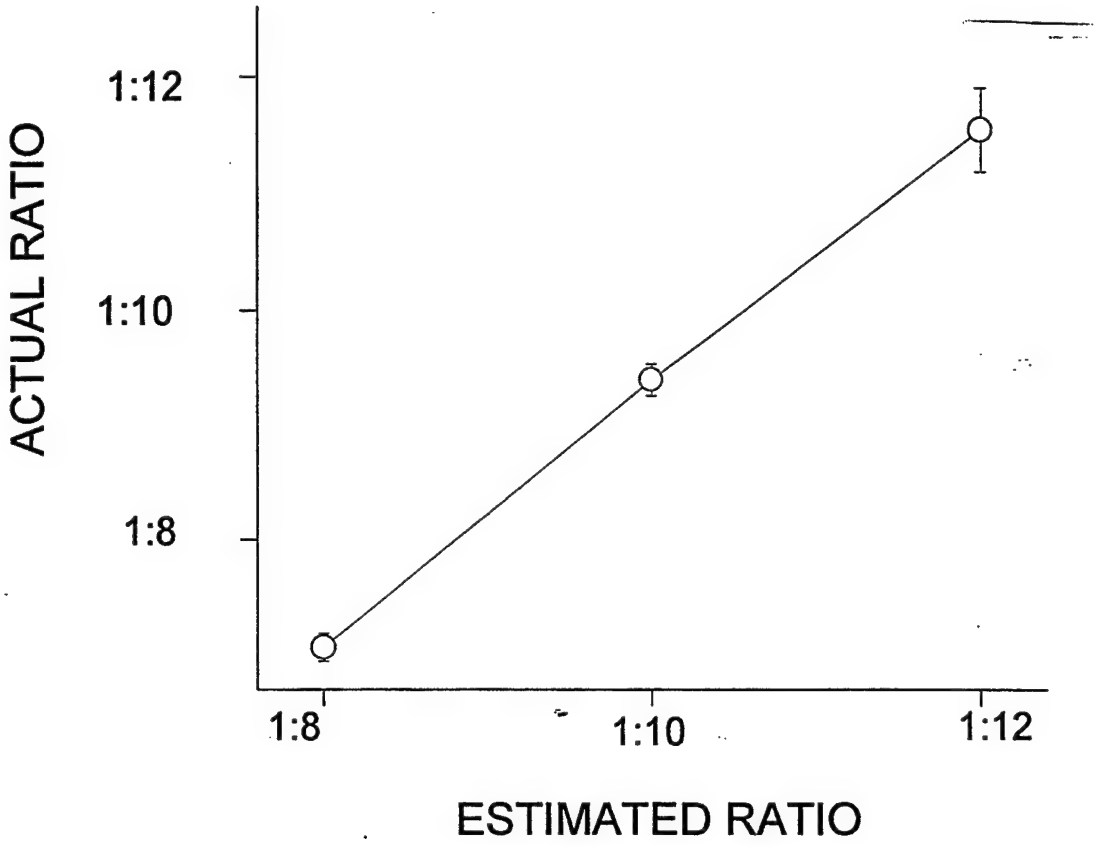

FIGURE 1

FIGURE 2

Platelet aggregates were determined by flow cytometry, using log forward light scatter (LFS) as a measure of platelet size by method 1, Percent positive LFS events using the peripheral blood sample as the control. Only those particles that bound a GPIIIa-specific antibody (platelet-specific) were analyzed. All data are mean ± S.E.M., n = 5. * Indicates $p < 0.05$ (by non-paired student's *t* test for 1:10 ACD:blood collection ratio compared to 1:8). + Indicates $p < 0.05$ (by non-paired student's *t* test for 1:12 ACD:blood collection ratio compared to 1:8).

(r) = rotated, (nr) = non-rotated, (nr→r) = non-rotated for first 4 hours and then rotated for 20 hours.

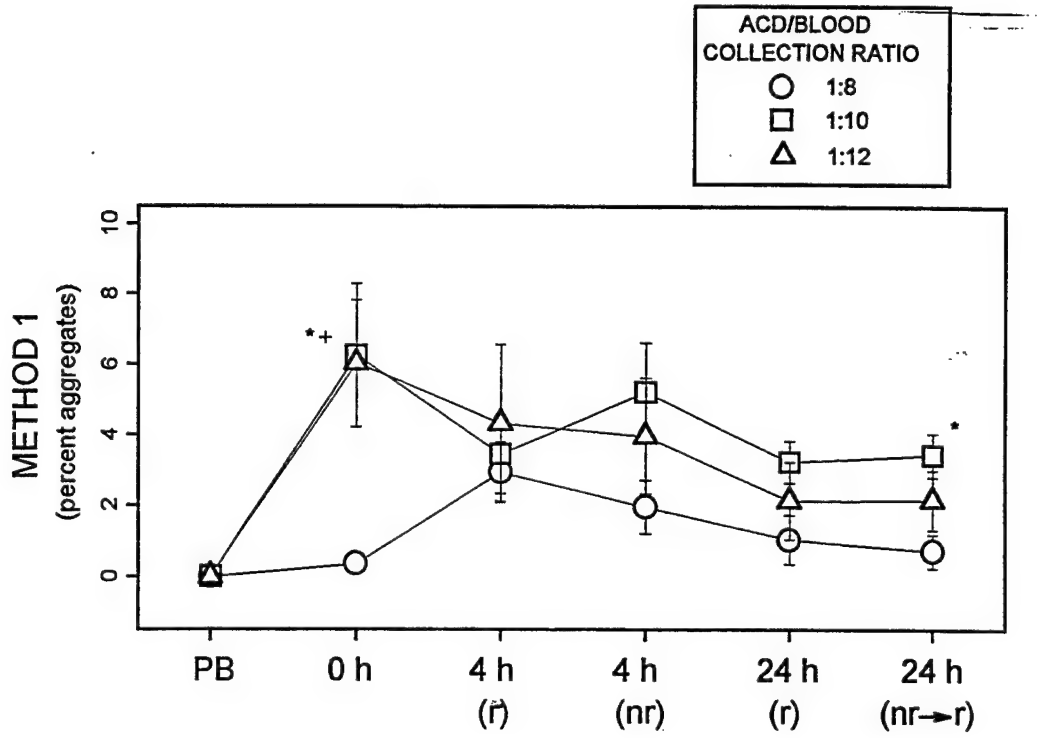
ACD/BLOOD
COLLECTION RATIO
1:8
1:10
1:12
METHOD 1
(percent aggregates)
0
2
4
6
8
10
*+
*
PB
0 h
4 h
(r)
4 h
(nr)
24 h
(r)
24 h
(nr→r)

FIGURE 2

FIGURE 3

Platelet aggregates were determined by flow cytometry, using log forward light scatter (LFS) as a measure of platelet size by method 2, Overton subtraction of the peripheral blood control LFS histogram from the test histogram. Only those particles that bound a GPIIIa-specific antibody (platelet-specific) were analyzed. All data are mean ± S.E.M., n = 5. * Indicates $p < 0.05$ (by non-paired student's *t* test for 1:10 ACD:blood collection ratio compared to 1:8). + Indicates $p < 0.05$ (by non-paired student's *t* test for 1:12 ACD:blood collection ratio compared to 1:8).
(r) = rotated, (nr) = non-rotated, (nr→r) = non-rotated for first 4 hours and then rotated for 20 hours.

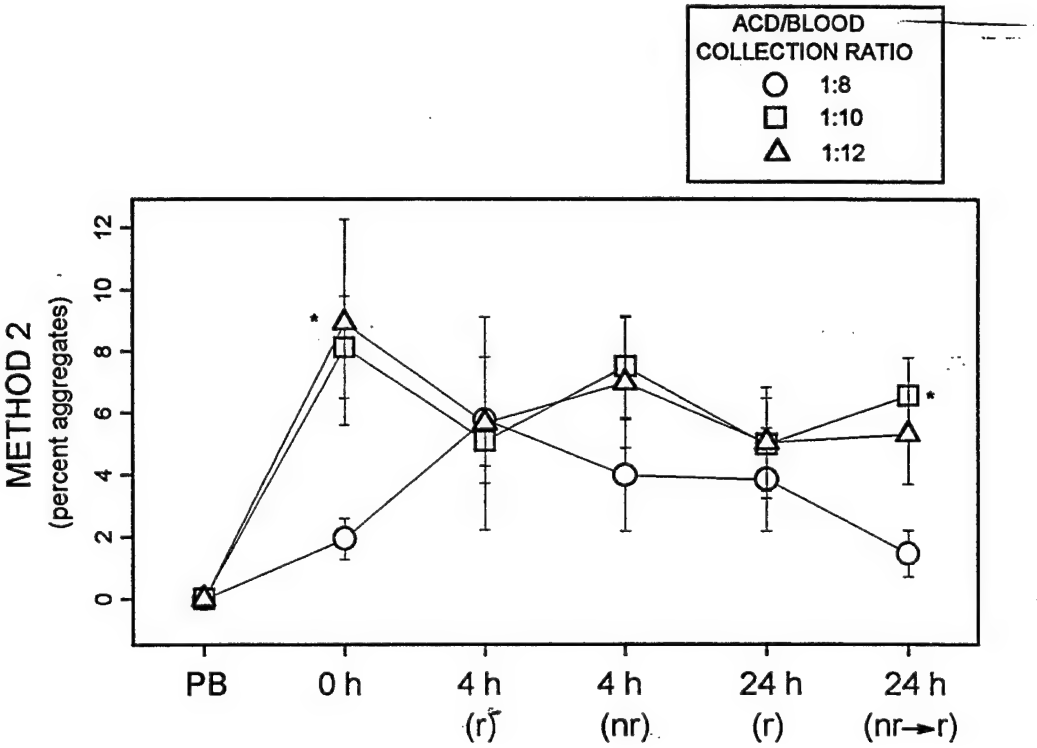
ACD/BLOOD
COLLECTION RATIO
1:8
1:10
1:12
METHOD 2
(percent aggregates)
0
2
4
6
8
10
12
*
*
PB
0 h
4 h
(r)
4 h
(nr)
24 h
(r)
24 h
(nr→r)

FIGURE 3

FIGURE 4

Platelet aggregates were determined by flow cytometry, using log forward light scatter (LFS) as a measure of platelet size by method 3, Mean linear forward light scatter. The peripheral blood control was assigned 100 units. Only those particles that bound a GPIIIa-specific antibody (platelet-specific) were analyzed. All data are mean ± S.E.M., n = 5. * Indicates $p < 0.05$ (by non-paired student's *t* test for 1:10 ACD:blood collection ratio compared to 1:8). + Indicates $p < 0.05$ (by non-paired student's *t* test for 1:12 ACD:blood collection ratio compared to 1:8).

(r) = rotated, (nr) = non-rotated, (nr→r) = non-rotated for first 4 hours and then rotated for 20 hours.

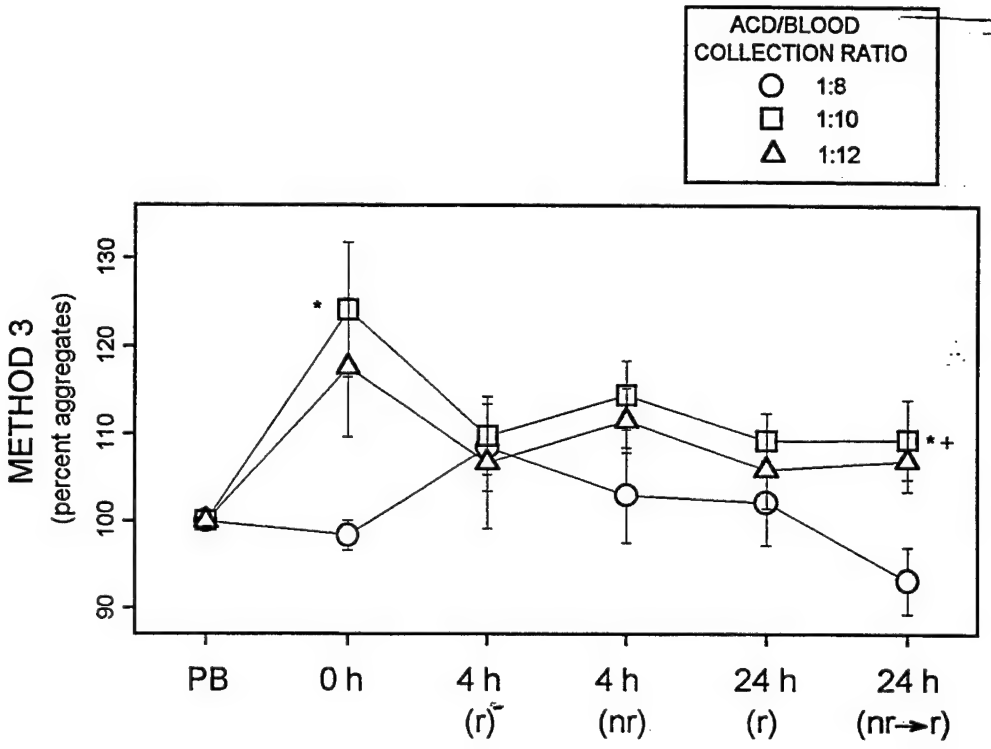
ACD/BLOOD
COLLECTION RATIO
1:8
1:10
1:12
METHOD 3
(percent aggregates)
130
120
110
100
90
*
*+
PB
0 h
4 h
(r)
4 h
(nr)
24 h
(r)
24 h
(nr→r)

FIGURE 4

FIGURE 5

Platelet-derived microparticles were determined by flow cytometry, using log forward light scatter as a measure of platelet size. Only those particles that bound a GPIIIa-specific antibody (platelet-specific) were analyzed. For analysis of the number of platelet-derived microparticles the control peripheral blood sample region was set at 4%. This analysis region was applied to all subsequent samples. Data are mean ± S.E.M., n = 4.

(r) = rotated, (nr) = non-rotated, (nr→r) = non-rotated for first 4 hours and then rotated for 20 hours.

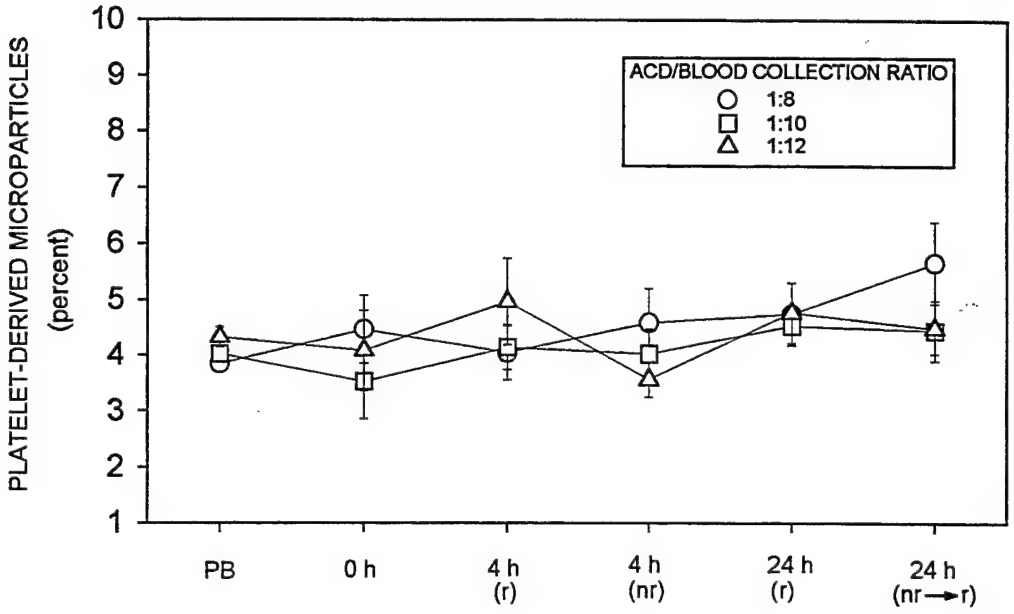
PLATELET-DERIVED MICROPARTICLES
(percent)
10
9
8
7
6
5
4
3
2
1
ACD/BLOOD COLLECTION RATIO
1:8
1:10
1:12
PB
0 h
4 h
(r)
4 h
(nr)
24 h
(r)
24 h
(nr→r)

FIGURE 5

FIGURE 6.

Representative scatter plots (log forward light scatter vs. log orthogonal light scatter) from one donor at the three ACD:blood collection ratios and all storage time points.

FIGURE 6

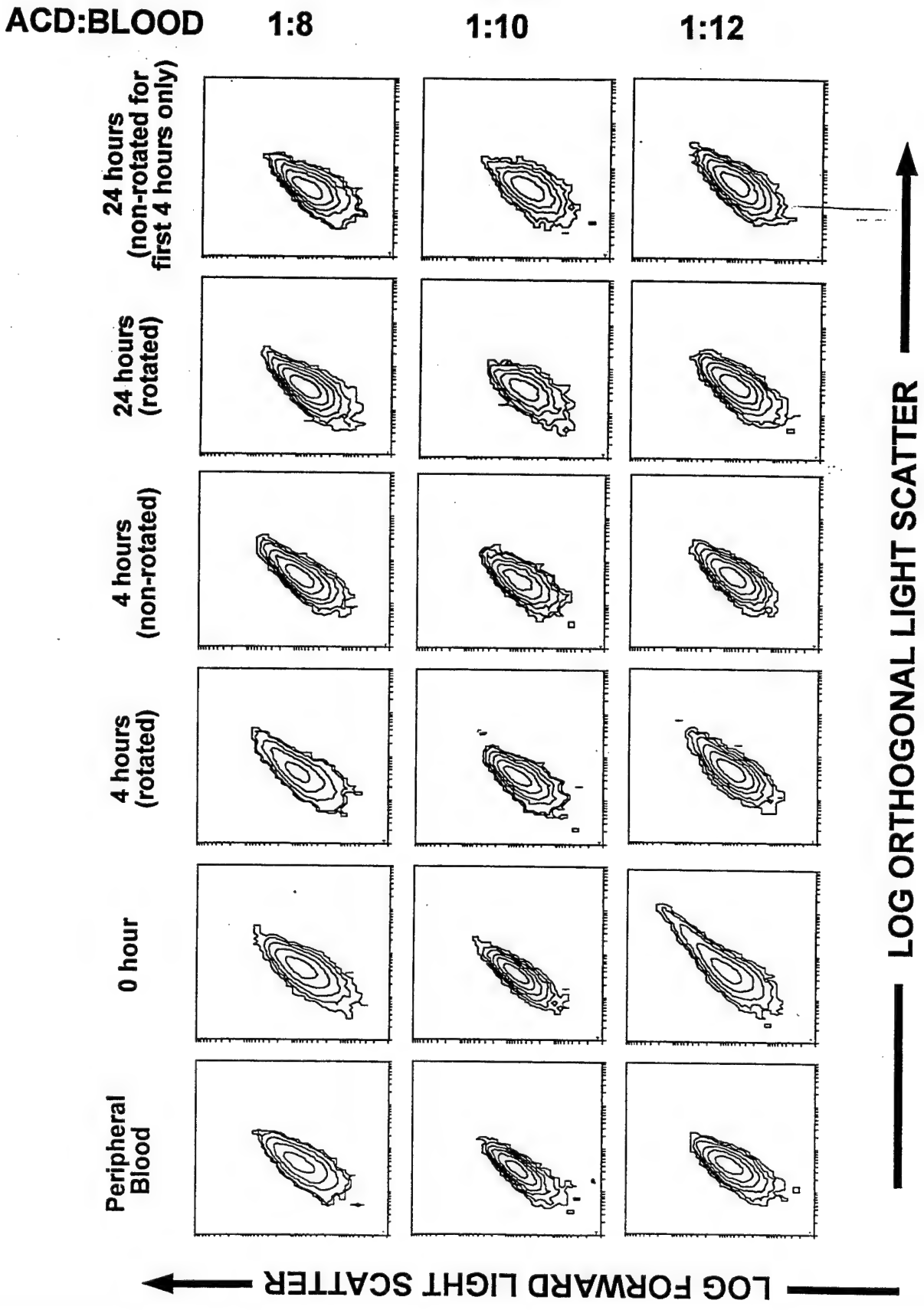
ACD:BLOOD
1:8
1:10
1:12
24 hours (non-rotated for first 4 hours only)
24 hours (rotated)
4 hours (non-rotated)
4 hours (rotated)
0 hour
Peripheral Blood
LOG ORTHOGONAL LIGHT SCATTER
LOG FORWARD LIGHT SCATTER

FIGURE 7A and 7B

Scatter plots (log forward light scatter vs. log orthogonal light scatter) comparing peripheral blood to the 0 hour sampling time point. Data are shown for all 5 donors at the three ACD:blood collection ratios. Donors 1 through 3 are shown in figure 7a and 4 through 5 in figure 7b.

FIGURE 7A

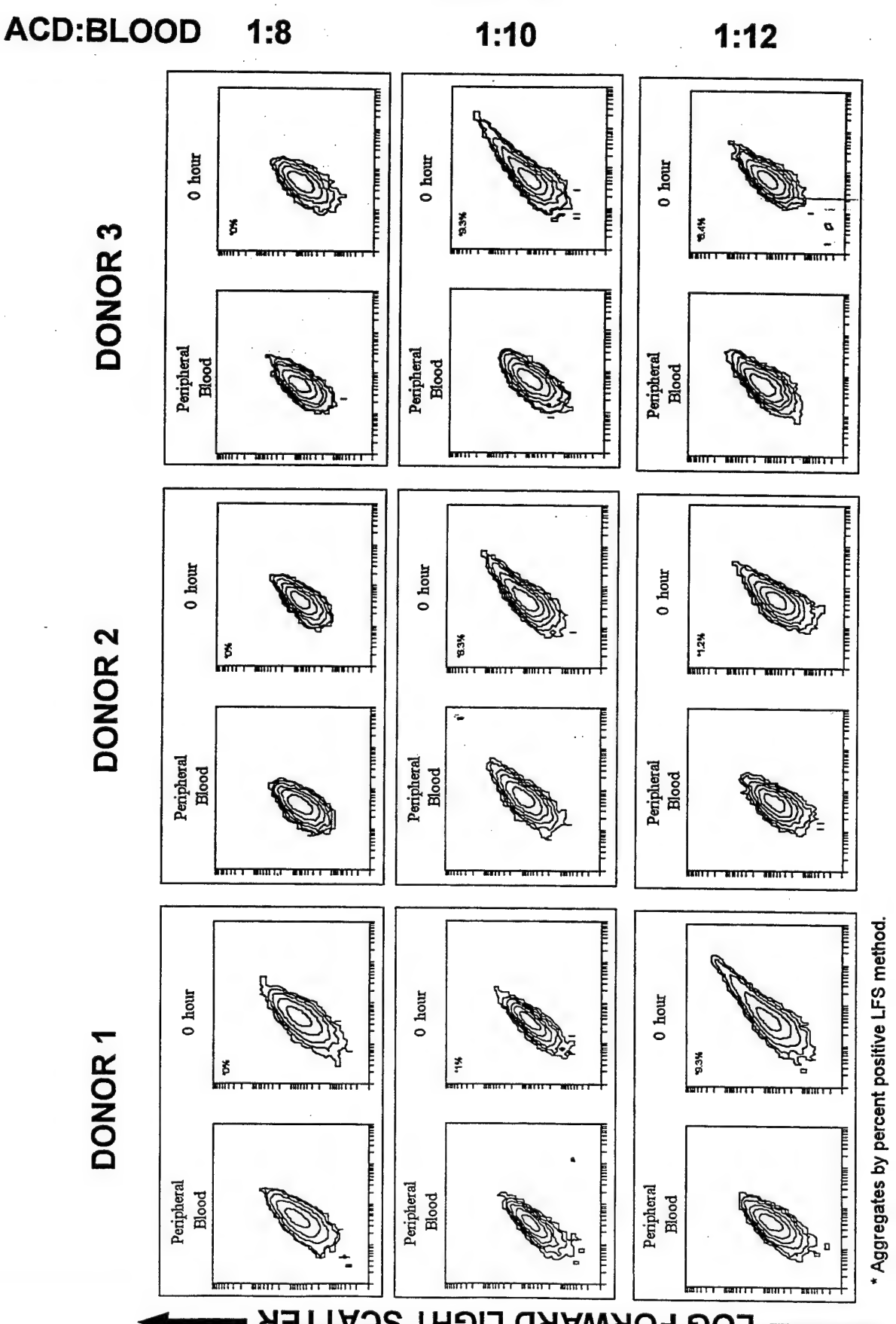
ACD:BLOOD
1:8
1:10
1:12
DONOR 1
DONOR 2
DONOR 3
Peripheral Blood
0 hour
LOG FORWARD LIGHT SCATTER
LOG ORTHOGONAL LIGHT SCATTER
* Aggregates by percent positive LFS method.

FIGURE 7B

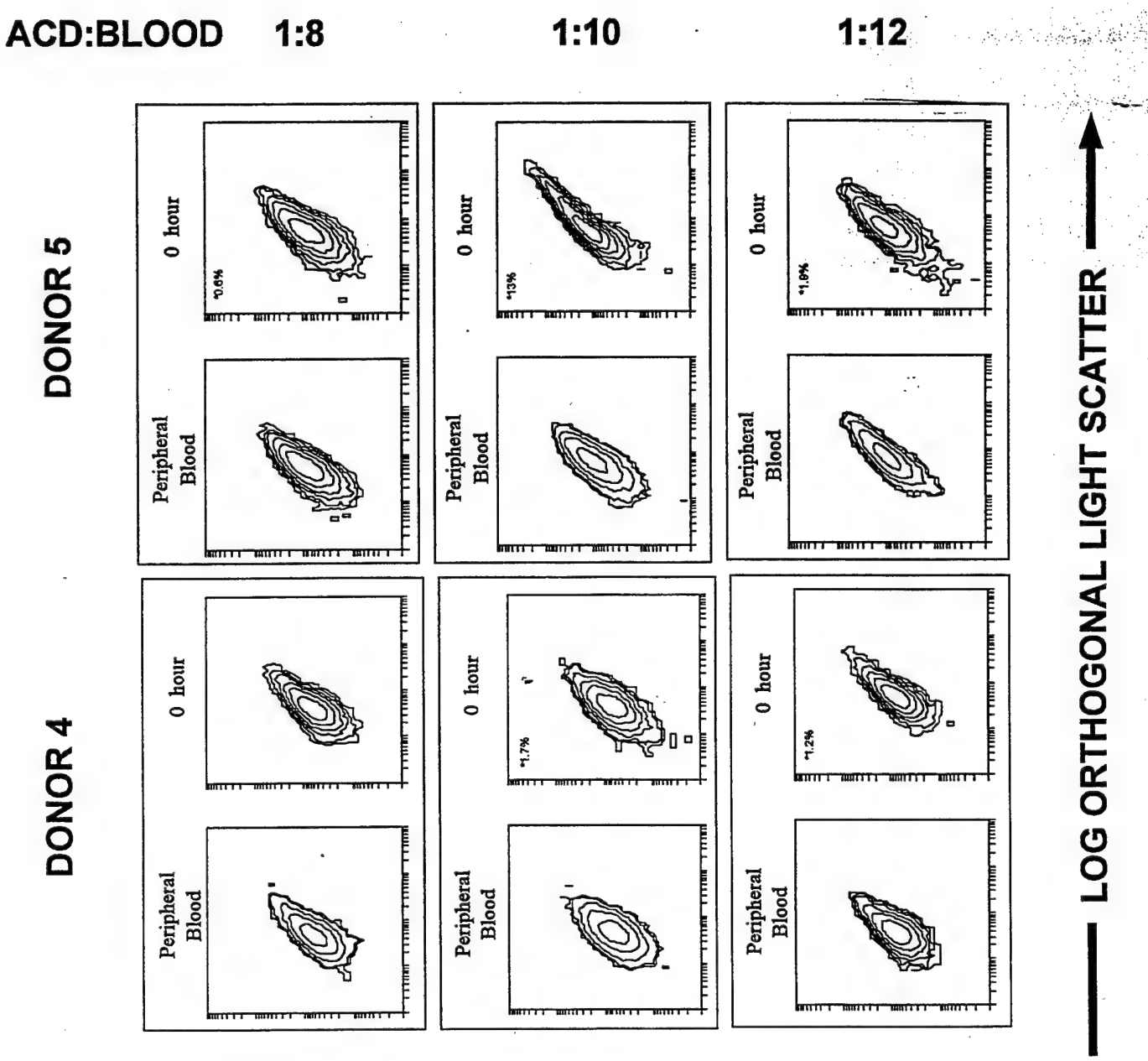
ACD:BLOOD
1:8
1:10
1:12
DONOR 5
DONOR 4
Peripheral Blood
0 hour
0.6%
1.3%
1.8%
1.7%
1.2%
LOG FORWARD LIGHT SCATTER
LOG ORTHOGONAL LIGHT SCATTER

FIGURE 8

Platelet surface GPIb as determined by flow cytometry. Samples were incubated (22°C, 15 minutes) with the following agonists: thrombin 2U/mL with 2.5 mM gly-pro-arg-pro (an inhibitor of fibrin polymerization), a combination of ADP 10 µM and arachidonic acid 50 µg/mL, or buffer only. The fluorescence intensity of peripheral blood samples in the absence of agonist was assigned 100%. Data are mean ± S.E.M., n = 4.

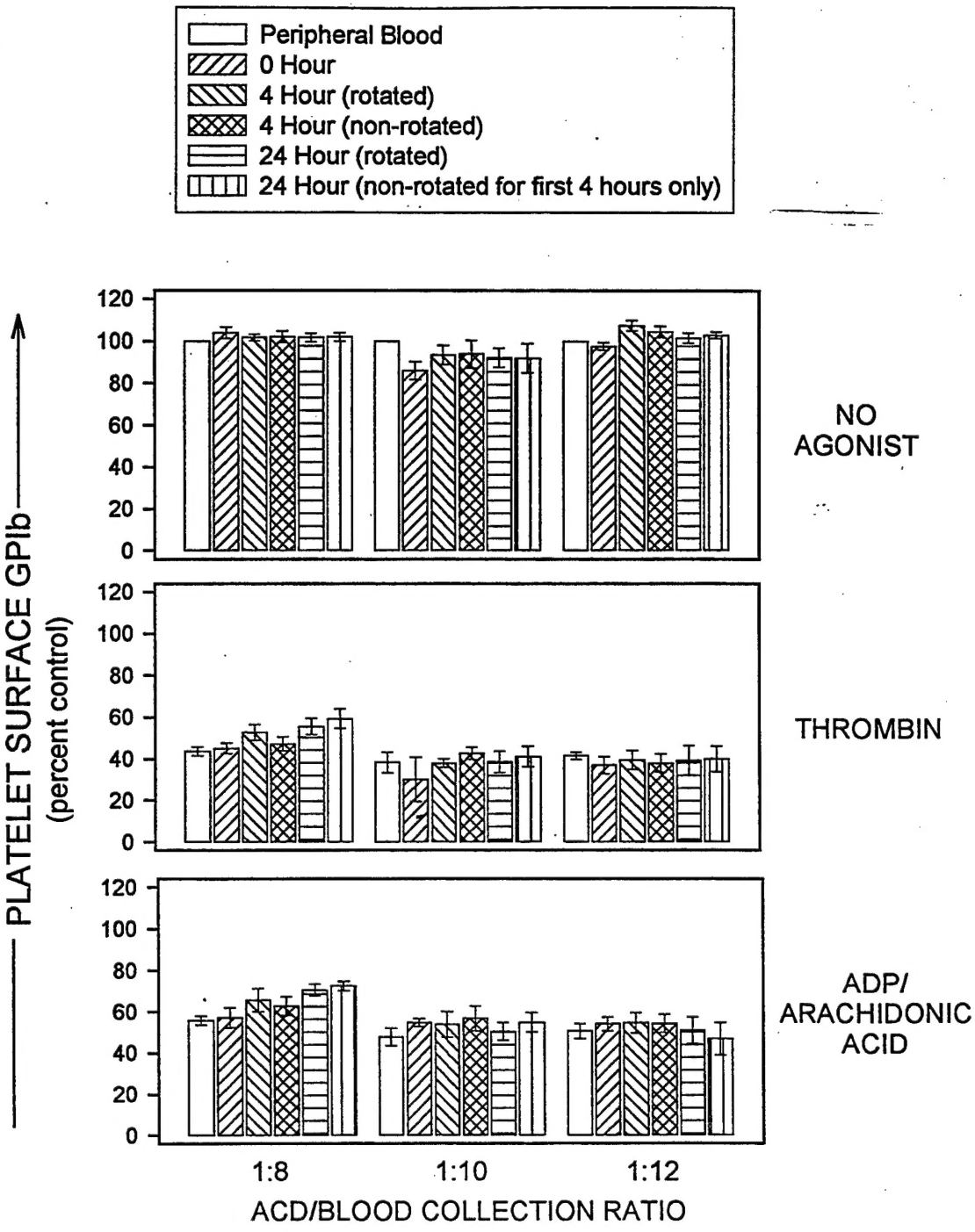
Peripheral Blood
0 Hour
4 Hour (rotated)
4 Hour (non-rotated)
24 Hour (rotated)
24 Hour (non-rotated for first 4 hours only)
PLATELET SURFACE GPIb
(percent control)
120
100
80
60
40
20
0
NO AGONIST
THROMBIN
ADP/ ARACHIDONIC ACID
1:8
1:10
1:12
ACD/BLOOD COLLECTION RATIO

FIGURE 8

FIGURE 9

Platelet surface P-selectin as determined by flow cytometry. Samples were incubated (22°C, 15 minutes) with the following agonists: thrombin 2U/mL with 2.5 mM gly-pro-arg-pro (an inhibitor of fibrin polymerization), a combination of ADP 10 µM and arachidonic acid 50 µg/mL, or buffer only. The fluorescence intensity of peripheral blood samples incubated with maximal thrombin (2 U/mL) was assigned 100%. Data are mean ± S.E.M., n = 4.

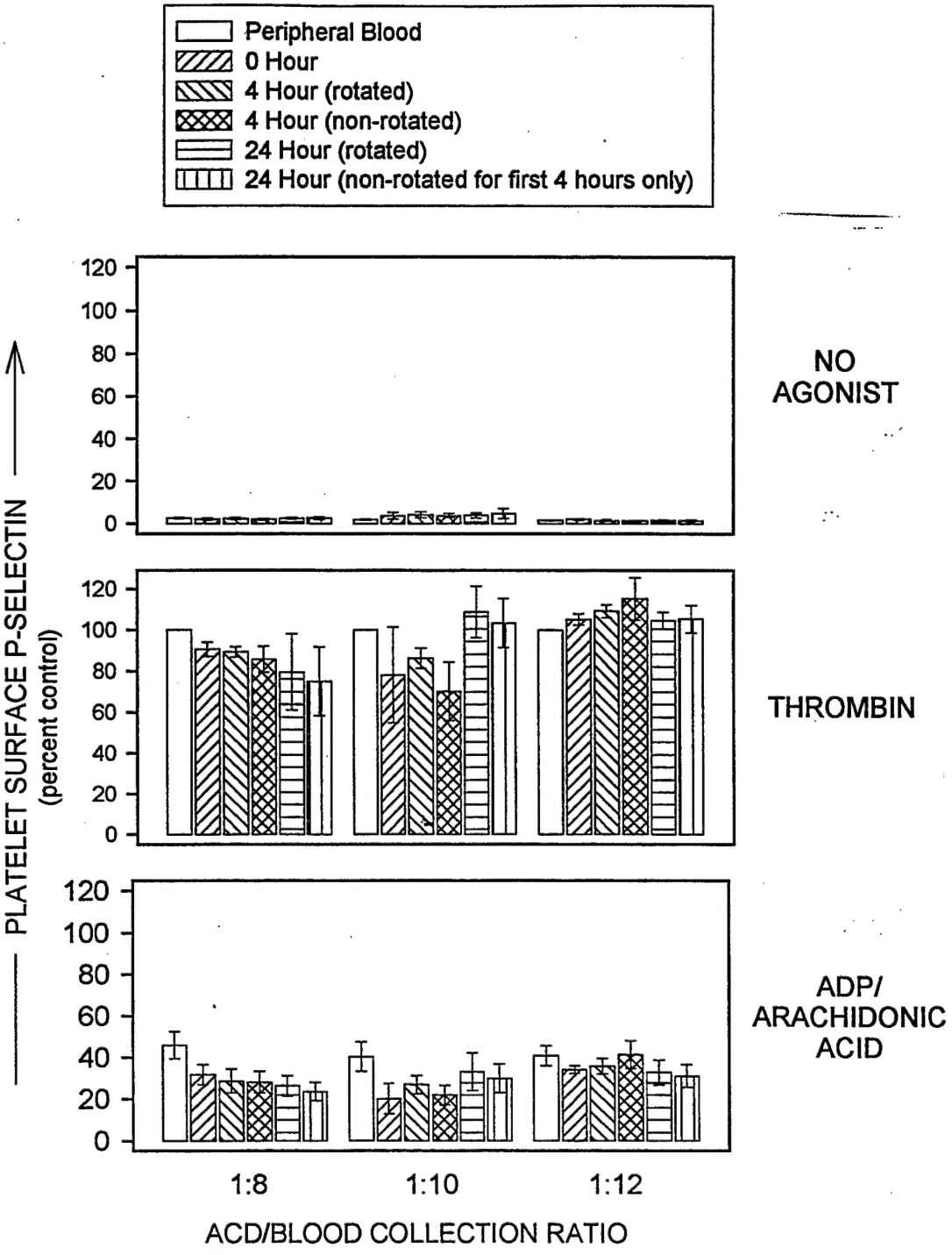
Peripheral Blood
0 Hour
4 Hour (rotated)
4 Hour (non-rotated)
24 Hour (rotated)
24 Hour (non-rotated for first 4 hours only)
PLATELET SURFACE P-SELECTIN
(percent control)
120
100
80
60
40
20
0
NO AGONIST
THROMBIN
ADP/ ARACHIDONIC ACID
1:8
1:10
1:12
ACD/BLOOD COLLECTION RATIO

FIGURE 9